

University of Dundee

DOCTOR OF MEDICINE

Allopurinol Regresses Left Ventricular Hypertrophy in Patients with Type 2 Diabetes.

Szwejkowski, Benjamin

Award date:
2014

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

DOCTOR OF MEDICINE

Allopurinol Regresses Left Ventricular Hypertrophy in Patients with Type 2 Diabetes.

Benjamin Szwejkowski

2014

University of Dundee

Conditions for Use and Duplication

Copyright of this work belongs to the author unless otherwise identified in the body of the thesis. It is permitted to use and duplicate this work only for personal and non-commercial research, study or criticism/review. You must obtain prior written consent from the author for any other use. Any quotation from this thesis must be acknowledged using the normal academic conventions. It is not permitted to supply the whole or part of this thesis to any other person or to post the same on any website or other online location without the prior written consent of the author. Contact the Discovery team (discovery@dundee.ac.uk) with any queries about the use or acknowledgement of this work.

Allopurinol Regresses Left Ventricular Hypertrophy in Patients with Type 2 Diabetes.

Benjamin Robert Szwejkowski

MBChB (Dundee), MRCP (UK)

Degree of Doctor of Medicine

University of Dundee

2014

CONTENTS PAGE

| | |
|--------------------|----|
| Contents | 2 |
| Tables and Figures | 6 |
| Abbreviations | 8 |
| Acknowledgements | 10 |
| Declaration | 11 |
| Thesis Summary | 12 |

Chapter 1: Introduction

| | | |
|------|--|----|
| 1. | The Culprit of Left Ventricular Hypertrophy in Diabetes | 14 |
| a. | The Increasing Problem of Type 2 Diabetes | 14 |
| i. | Definition and Diagnosis of Diabetes Mellitus | 14 |
| ii. | The Incidence and Burden of Diabetes Mellitus | 15 |
| b. | Diabetes and Cardiovascular Disease | 17 |
| 2. | Left Ventricular Hypertrophy in Patients with Diabetes | 19 |
| a. | Definitions of Left Ventricular Hypertrophy | 19 |
| i. | Electrocardiographic Criteria for Left Ventricular Hypertrophy | 19 |
| ii. | Echocardiographic Criteria for Left Ventricular Hypertrophy | 20 |
| iii. | Cardiac MRI Criteria for Left Ventricular Hypertrophy | 20 |
| b. | Prevalence of Left Ventricular Hypertrophy | 26 |
| c. | Increased Cardiovascular Risk Associated with Left Ventricular Hypertrophy | 29 |
| d. | Left Ventricular Hypertrophy and its Association with Increased Cardiovascular Risk | 31 |
| i. | Myocardial ischaemia and left ventricular hypertrophy | 31 |
| ii. | Arrhythmias and Left Ventricular Hypertrophy | 34 |
| iii. | Left Atrial Size and Left Ventricular Hypertrophy | 35 |
| iv. | Atrial Fibrillation in Diabetes | 36 |
| v. | Summary | 39 |
| 3. | The Development of Left Ventricular Hypertrophy | 40 |
| a. | Blood Pressure and Obesity | 40 |
| b. | Oxidative stress in Diabetes | 42 |
| i. | The Concept of Oxidative Stress | 42 |
| ii. | Oxidative Stress Causing Left Ventricular Hypertrophy | 45 |
| iii. | Diabetes as a Source of Oxidative Stress | 47 |
| c. | Endothelial function | 47 |
| i. | Principles of Endothelial Function | 47 |
| ii. | Endothelial dysfunction in diabetes | 51 |
| iii. | Augmentation Index | 52 |
| iv. | Pulse Wave Velocity | 56 |
| v. | Flow Mediated Dilatation and Prognostic Value of Endothelial Dysfunction | 56 |
| d. | Direct Affects of Glucose and Insulin on the Development of Left Ventricular Hypertrophy | 60 |
| e. | Other Causes of Left Ventricular Hypertrophy | 64 |
| i. | Genetic Factors | 64 |
| ii. | Renin Angiotensin System | 64 |
| f. | Brain Natriuretic Peptide in Left Ventricular Hypertrophy | 65 |

| | | |
|------|--|-----|
| 4. | Treating Left Ventricular Hypertrophy | 66 |
| a. | Benefits and Established ways of Regressing Left Ventricular Hypertrophy | 66 |
| i. | Losartan Intervention For Endpoint Reduction in Hypertension (LIFE) Study | 68 |
| ii. | The Heart Outcomes Prevention Evaluation (HOPE) Study | 70 |
| iii. | Regressing Left Ventricular Hypertrophy in the Context of Normal Blood Pressure | 71 |
| iv. | Non-Pharmacological Ways of Regressing Left Ventricular Hypertrophy | 72 |
| b. | Potential Ways of Regressing Left Ventricular Hypertrophy | 74 |
| 5. | Xanthine Oxidase and Allopurinol | 75 |
| a. | History of the Xanthine Oxidase Inhibitor Allopurinol | 75 |
| b. | Xanthine Oxidase | 77 |
| i. | Structure | 77 |
| ii. | Actions | 79 |
| iii. | Distribution | 81 |
| iv. | Possible Pathophysiological Roles of XOR | 83 |
| c. | Biochemistry of Allopurinol | 85 |
| d. | Clinical Pharmacokinetics of Allopurinol | 86 |
| e. | Dose response studies of allopurinol | 88 |
| f. | Clinical Pharmacodynamics of Allopurinol | 89 |
| g. | Allopurinol as an Antioxidant | 89 |
| i. | Allopurinol may Improve Mechanoenergetic Uncoupling | 90 |
| ii. | Allopurinol as a Free Radical Scavenger in Animal Models | 91 |
| iii. | Evidence Allopurinol may be Beneficial in IR Injury | 92 |
| iv. | Allopurinol may Improve Left Ventricular Remodelling after Myocardial Infarction | 94 |
| h. | Allopurinol Improves Endothelial Function in Various Cardiovascular Disorders | 95 |
| i. | Allopurinol Improves Augmentation Index and Brain Natriuretic Peptide | 98 |
| j. | Effects of Allopurinol in Ischaemic Stroke | 98 |
| k. | Effects of Allopurinol on Blood Pressure | 99 |
| l. | Allopurinol May Regress Left Ventricular Hypertrophy | 99 |
| m. | Dosing of Allopurinol | 100 |
| 6. | Aims | 102 |

Chapter 2: Methods

| | | |
|----|--------------------------------------|-----|
| 1. | Approvals and Trial Registration | 103 |
| 2. | Study Design | 104 |
| a. | Study participants | 104 |
| b. | Exclusion Criteria | 105 |
| c. | Allopurinol: Dosing and Side Effects | 105 |
| d. | Study Visits and Drug Titration | 106 |
| e. | Cardiac Magnetic Resonance Imaging | 109 |
| f. | Flow Mediated Dilatation | 110 |
| g. | Applanation Tonometry | 111 |
| h. | Laboratory Methods | 112 |
| i. | Biochemistry and Haematology Tests | 112 |

| | | |
|------|------------------------------------|-----|
| ii. | Brain Natriuretic Peptide | 112 |
| iii. | Uric Acid | 113 |
| iv. | Insulin | 113 |
| v. | Oxidised LDL | 113 |
| i. | Drug Manufacture and Randomisation | 114 |
| j. | Statistical Analysis | 114 |

Chapter 3: Results

| | | |
|----|--|-----|
| 1. | Study Recruitment | 116 |
| 2. | Baseline Characteristics | 119 |
| 3. | Changes in Measured Parameters | 122 |
| 4. | Changes in Measured Parameters for Above and Below Median LVM and LVMI | 127 |
| 5. | Correlations | 136 |
| 6. | Important Drug Changes over Nine-Month Study Period | 138 |

Chapter 4: Discussion

| | | |
|-----|---|-----|
| 1. | Blood Pressure | 140 |
| 2. | Prognostic Benefits of Regressing LVH | 141 |
| 3. | Magnitude of Changes in Left Ventricular Mass | 143 |
| 4. | Other Studies Regressing Left Ventricular Hypertrophy Using Allopurinol | 144 |
| 5. | Reducing Cardiovascular Disease Further in Diabetes | 145 |
| 6. | Possible Mechanisms Why Allopurinol Reduces Left Ventricular Mass | 146 |
| 7. | Allopurinol and its Effects on BNP | 150 |
| 8. | Effects of Allopurinol on other Cardiac MRI Parameters | 151 |
| 9. | Association of Uric Acid with Cardiovascular Disease | 152 |
| 10. | Dose Response of Allopurinol | 153 |
| 11. | Effects of Allopurinol on Hyperglycaemia | 154 |
| 12. | Side Effects and Tolerance of Allopurinol | 155 |
| 13. | Clinical Relevance | 156 |
| 14. | Limitations | 158 |
| 15. | Future Research | 159 |
| a. | Cardiovascular Outcomes | 159 |
| b. | Myocardial Fibrosis | 159 |

| | | |
|-----|---------------------------------------|-----|
| c. | LA size | 159 |
| 16. | Publications and Poster Presentations | 161 |
| a. | Prizes | 161 |
| b. | Publications | 161 |
| c. | Poster Presentations | 162 |
| 17. | References | 163 |
| 18. | Appendices | 180 |
| a. | Consent form | 181 |
| b. | Patient Information Sheet | 182 |
| c. | GP Letter | 188 |
| d. | Case Report Form | 190 |

Tables and Figures

Chapter 1

Tables

| | |
|--|----|
| <i>Table 1.1.1. Diagnostic values for diagnosing diabetes</i> | 14 |
| <i>Table 1.1.2. Prevalence of diabetes in the UK 2005/06</i> | 16 |
| <i>Table 1.2.1. The normal values for LVM and LVM indexed to body surface area and height using SSFP MRI</i> | 22 |
| <i>Table 1.2.2. The normal values for LVM and LV mass indexed to body surface area, height and body mass index using FLASH MRI</i> | 23 |
| <i>Table 1.2.3. Summary of the predicted lower, mean and upper limits for normal LV parameters in males and females of different ages</i> | 25 |
| <i>Table 1.2.4. Prevalence of LVH as defined by the new ASE guidelines</i> | 27 |
| <i>Table 1.2.5. Comparison of the association of AF with Diabetes from the major published studies expressed as odds and hazard ratios</i> | 38 |
| <i>Table 1.5.1. Regulation of XOR gene expression</i> | 82 |
| <i>Table 1.5.2. Oxypurinol dose response table</i> | 88 |

Figures

| | |
|--|----|
| <i>Figure 1.2.1. Summary of mean risk ratio for available studies of baseline LVH for CV events</i> | 30 |
| <i>Figure 1.3.1. The principle reactions involved in the generation and degradation of hydrogen peroxide</i> | 43 |
| <i>Figure 1.3.2. The basic pathway for the generation of ROS</i> | 44 |
| <i>Figure 1.3.3. Role of XO in pressure overload and heart failure.</i> | 44 |
| <i>Figure 1.3.4. ROS mediated myocyte hypertrophy</i> | 46 |
| <i>Figure 1.3.5. Pathophysiological effects of oxidative stress in LVH and heart failure</i> | 46 |
| <i>Figure 1.3.6. The Nitric Oxide Signalling Pathway</i> | 49 |
| <i>Figure 1.3.7. Central Pulse Pressure Waveform or Augmentation Index</i> | 54 |
| <i>Figure 1.3.8. Effects of changes in LV workload on Aix</i> | 55 |
| <i>Figure 1.3.9. Potential mechanisms by which insulin resistance are associated with left ventricular hypertrophy</i> | 62 |
| <i>Figure 1.3.10. Summary of the possible mechanisms for metabolic adaptation and maladaptation of the heart</i> | 63 |
| <i>Figure 1.5.1 Purine degradation pathway</i> | 76 |
| <i>Figure 1.5.2. A: Molybdopterin and B: Molybdenum co-factor (Mo-co)</i> | 78 |
| <i>Figure 1.5.3. Crystal structure of xanthine dehydrogenase</i> | 78 |
| <i>Figure 1.5.4. Formation of reactive oxygen species and NADH from the XOR catalysed oxidation of xanthine and hypoxanthine</i> | 79 |
| <i>Figure 1.5.5. Mechanism of XOR reaction with Xanthine</i> | 80 |
| <i>Figure 1.5.6. XOR catalysed production of peroxynitrite</i> | 81 |
| <i>Figure 1.5.7. Mechanism of ROS during ischaemia reperfusion injury</i> | 84 |
| <i>Figure 1.5.8. Chemical structures of allopurinol and oxypurinol</i> | 85 |

Chapter 2

Figures

| | |
|--|-----|
| <i>Figure 2.2.1. Outline of Study Visits</i> | 108 |
| <i>Figure 2.2.2. Typical endocardial and epicardial border region-of-interest contouring of a set of images from the study</i> | 110 |

Chapter 3

Tables

| | |
|---|-----|
| <i>Table 3.2.1. Baseline Characteristics</i> | 120 |
| <i>Table 3.3.1. Changes in MRI parameters (Over 9 month study period)</i> | 122 |
| <i>Table 3.3.2. Output from multivariate analysis using co-variables of baseline BP, change in BP and prescription of ACE inhibitor or ARBs</i> | 123 |
| <i>Table 3.3.3. Change in blood parameters (Over 9 month study period)</i> | 123 |
| <i>Table 3.3.4. Change in parameters of endothelial function and blood pressure</i> | 126 |
| <i>Table 3.4.1. Changes in measured parameters analysed as sub-groups of above and below median LVM</i> | 129 |
| <i>Table 3.4.2. Changes in measured parameters analysed as sub-groups of above and below median LVMI</i> | 129 |
| <i>Table 3.4.3. Sub-group analysis of above and below median baseline LVM for change in blood parameters (Over 9 month study period)</i> | 130 |
| <i>Table 3.4.4. Sub-group analysis of above and below median baseline LVMI for change in blood parameters (Over 9 month study period)</i> | 131 |
| <i>Table 3.4.5. Sub-group analysis of above and below median baseline LVM for change in parameters of endothelial function and blood pressure</i> | 132 |
| <i>Table 3.4.6. Sub-group analysis of above and below median baseline LVM for change in parameters of endothelial function and blood pressure</i> | 133 |
| <i>Table 3.5.1. Correlation of LVM and LVMI with measured parameters</i> | 137 |
| <i>Table 3.6.2. Important drug Changes over nine-month study period</i> | 138 |

Figures

| | |
|--|-----|
| <i>Figure 3.1.1. Study recruitment CONSORT diagram</i> | 118 |
| <i>Figure 3.3.1. Diagrams of changes in (1) LVM and (2) LVMI</i> | 124 |
| <i>Figure 3.4.1 Graphs of above and below median LVM changes in (1) LVM and (2) LVMI</i> | 134 |
| <i>Figure 3.4.2. Graphs of above and below median LVMI changes in (1) LVM and (2) LVMI</i> | 135 |

Abbreviations

| | |
|----------------|--|
| ABPM | Ambulatory Blood Pressure Monitoring |
| ACE inhibitors | Angiotensin-Converting Enzyme Inhibitors |
| AF | Atrial Fibrillation |
| AIx | Augmentation Index |
| ARB | Angiotensin Receptor Blockers |
| BMI | Body Mass Index |
| BNP | Brain Natriuretic Peptide |
| BP | Blood Pressure |
| BSA | Body Surface Area |
| CAD | Coronary Artery Disease |
| CHD | Coronary Heart Disease |
| CI | Confidence Interval |
| CMR | Cardiac Magnetic Resonance Imaging |
| CO | Cardiac Output |
| CV | Cardiovascular |
| ECG | Electrocardiogram |
| Echo | Echocardiogram |
| EDV | End-Diastolic Volume |
| EDHF | Endothelial Derived Hyperpolarising Factor |
| EDRF | Endothelial Derived Relaxing Factor |
| EF | Ejection Fraction |
| ESV | End-Systolic Volume |
| FLASH | Fast Gradient Echo Imaging |
| FMD | Flow Mediated Dilatation |
| HOPE | Heart Outcomes Prevention Evaluation |
| HS Troponin T | High Sensitivity Troponin T |
| IGF-1 | Insulin Like Growth Factor |
| IHD | Ischaemic Heart Disease |
| IR | Ischaemia Reperfusion Injury |
| LA | Left Atrium |
| LIFE | The Losartan Intervention For Endpoint reduction in hypertension |
| LVH | Left Ventricular Hypertrophy |
| LVM | Left Ventricular Mass |
| LVMI | Left Ventricular Mass Index to Body Surface Area |
| NO | Nitric Oxide |
| NOS | Nitric Oxide Synthase |
| OR | Odds Ratio |
| OS | Oxidative Stress |
| PCR | Protein:Creatinine Ratio |
| PVD | Peripheral Vascular Disease |
| PWA | Pulse Wave Analysis |
| PWV | Pulse Wave Velocity |
| RAS | Renin Angiotensin System |
| ROS | Reactant Oxygen Species |
| RR | Relative Risk |
| SOD | Superoxide Dismutase |
| SSFP | Steady State Free Precession |

| | |
|------|---------------------------|
| SV | Stroke Volume |
| T1DM | Type 1 Diabetes |
| T2DM | Type 2 Diabetes |
| WHO | World Health Organisation |
| VT | Ventricular Tachycardia |
| XDH | Xanthine Dehydrogenase |
| XO | Xanthine Oxidase |
| XOR | Xanthine Oxidoreductase |

Acknowledgments

Firstly I would like to thank Professor Struthers for giving me the opportunity to pursue this research. During my time in the Department, and whilst writing my thesis, he was very supportive and I feel I have had many opportunities and doors opened thanks to his support and advice.

Within the Clinical Pharmacology Department I was made to feel very welcome and would like to thank everyone who was present during my time there. In particular Steve McLaren provided invaluable technical support and Lesley McFarlane who analysed all the blood tests during the trial. Steve Gandy has spent many hours analysing and teaching me the 'ins and outs' of CMR analysis and without him and his skills at CMR then none of this study would be possible. Professor Morris taught and guided me through diabetes clinics throughout my time in the department, which allowed me to recruit patients as well.

I am grateful to Diabetes UK who funded this study.

My wife Shilpi has been, as ever, a great encouragement and support to me during this time. I started in research with no children and now have two called Nathan and Maia, my MD journey has been fulfilling both inside and outside of work. Finally thanks to my parents who have always stood by me and supported me in all aspects of my career.

Declaration

I declare solely myself authored this thesis during my time as a Clinical Research Fellow at the University of Dundee. All data was collected and analysed by myself, including the statistics, apart from the CMR analysis that was performed by Stephen Gandy and blood analysis that was performed by Leslie MacFarlane. I was employed as Diabetes UK Clinical Research Fellow within the Department of Heart and Lung Biology at the University of Dundee from February 2009 to February 2011.

I declare this work has not been submitted for a higher degree before. This work has been presented at various national and international meetings as acknowledged within the thesis. The main data from the study has been published in the Journal of the American College of Cardiology (JACC) in 2013.

Signed

Date

Thesis Summary

Left Ventricular Hypertrophy (LVH) is common in Type 2 Diabetes (T2DM) and despite optimal treatment of blood pressure can still persist. We know LVH is a cardiovascular (CV) risk factor in its own right and contributes to high CV event rates in patients with T2DM. Apart from hypertension, other factors contribute to the development of LVH in patients with T2DM, in particular oxidative stress (OS) has been implicated in LVH development. Allopurinol is a potent anti-oxidant, acting by blocking the enzyme Xanthine Oxidase, and has been previously shown to reduce vascular OS. Therefore the main aim of this thesis was to investigate whether allopurinol regresses LVH in patients with T2DM.

The trial design was a randomised, double blind, placebo controlled study in 66 patients with T2DM with echocardiographic evidence of LVH. Allopurinol 600mg/day or placebo was given for nine months over the study period. The primary outcome was reduction in left ventricular mass (LVM) as calculated by cardiac magnetic resonance imaging (CMR) at baseline and at nine months follow-up. The secondary end-points were change in flow mediated dilatation (FMD) and augmentation index (AIx).

Allopurinol significantly reduced absolute LVM ($-2.65 \pm 5.91\text{g}$ and placebo group $+1.21 \pm 5.10\text{g}$ ($p=0.012$)) and LVM indexed to body surface area ($-1.32 \pm 2.84\text{g/m}^2$ and placebo group $+0.65 \pm 3.07\text{g/m}^2$ ($p=0.017$)). When analysis was made of high and low baseline LVM then the effects of allopurinol were

exaggerated in the high LVM mass group. No significant change was seen in either FMD or AIx.

This thesis shows that allopurinol regresses LVM in patients with T2DM and LVH and controlled blood pressure. Regressing LVH has been shown previously to improve CV mortality and morbidity. Therefore allopurinol may become a useful therapy to reduce CV events in T2DM patients with LVH.

CHAPTER ONE

INTRODUCTION

1. The Culprit of Left Ventricular Hypertrophy in Diabetes

a. The Increasing Problem of Type 2 Diabetes

i. Definition and Diagnosis of Diabetes Mellitus

The World Health Organisation (WHO) in 1999 defined diabetes as (1): *'The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action, or both'*. The WHO consensus on the levels of blood glucose to diagnose diabetes are summarised in table 1.1.1.

| | Glucose concentration, mmol l ⁻¹ (mg dl ⁻¹) | | |
|--|--|-------------------|-------------------|
| | Whole blood | Capillary | Plasma* |
| | Venous | | Venous |
| Diabetes Mellitus: | | | |
| Fasting or | ≥ 6.1 (≥ 110) | ≥ 6.1 (≥ 110) | ≥ 7.0 (≥ 126) |
| 2-h post glucose load | ≥ 10.0 (≥ 180) | ≥ 11.1 (≥ 200) | ≥ 11.1 (≥ 200) |
| Impaired Glucose Tolerance (IGT): | | | |
| Fasting (if measured) and | < 6.1 (< 110) and | < 6.1 (< 110) and | < 7.0 (< 126) and |
| 2-h post glucose load | ≥ 6.7 (≥ 120) | ≥ 7.8 (≥ 140) | ≥ 7.8 (≥ 140) |
| Impaired Fasting Glycaemia (IFG): | | | |
| Fasting | ≥ 5.6 (≥ 100) and | ≥ 5.6 (≥ 100) and | ≥ 6.1 (≥ 110) and |
| | < 6.1 (< 110) | < 6.1 (< 110) | < 7.0 (< 126) |
| and (if measured) | | | |
| 2-h post glucose load | < 6.7 (< 120) | < 7.8 (< 140) | < 7.8 (< 140) |

* Corresponding values for capillary plasma are: for Diabetes Mellitus, fasting ≥ 7.0 (≥ 126), 2-h ≥ 12.2 (≥ 220); for Impaired Glucose Tolerance, fasting < 7.0 (< 126) and 2-h ≥ 8.9 (≥ 160) and < 12.2 (< 220); and for Impaired Fasting Glycaemia ≥ 6.1 (≥ 110) and < 7.0 (< 126) and if measured, 2-h < 8.9 (< 160).

For epidemiological or population screening purposes, the fasting or 2-h value after 75 g oral glucose may be used alone. For clinical purposes, the diagnosis of diabetes should always be confirmed by repeating the test on another day unless there is unequivocal hyperglycaemia with acute metabolic decompensation or obvious symptoms.

Glucose concentrations should not be determined on serum unless red cells are immediately removed, otherwise glycolysis will result in an unpredictable under-estimation of the true concentrations. It should be stressed that glucose preservatives do not totally prevent glycolysis. If whole blood is used, the sample should be kept at 0–4 °C or centrifuged immediately, or assayed immediately.

Table 1.1.1. Diagnostic values for diagnosing diabetes (1).

It is recommended the term 'insulin dependent diabetes mellitus' and 'non insulin dependent diabetes mellitus' are 'phased out' and the terms Type 1 Diabetes (T1DM) and Type 2 Diabetes (T2DM) should be used instead (1). The definition of type T1DM describes insulin deficiency due to loss of beta-islet cells from the pancreas, including autoimmunity and idiopathic causes. It does not include cases where the cause of beta islet cell destruction is attributable to a specific disease process or underlying disease process e.g. cystic fibrosis and fibrocalculous pancreatopathy (1). An important feature of T1DM is that they are prone to ketoacidosis. T2DM encompasses defects in insulin secretion and insulin resistance. It is the most common form of diabetes and it comprises almost 90% of all cases (1).

ii. The Incidence and Burden of Diabetes Mellitus

In 2006 Diabetes UK produced the report 'Diabetes: State of the Nations' where diabetes is described as 'one of the greatest health challenges facing the UK today' (2). The prevalence of diabetes has increased over the last nine years and it is estimated over two million people in the UK are living with diabetes, and it could be that up to half have not had their condition diagnosed (2). In 2006 the estimated prevalence of diabetes in Scotland was 3.4%, published in 'Diabetes: State of the Nations' (2). Gonzalez *et al* (2009) published data on the increase in incidence of T1DM and T2DM in the UK general population from 1996 to 2005 (3). Their data was sourced from the Health Improvement Network database, using data on 49,999 prevalent cases and 42,642 incident cases (T1DM n=1,256 and T2DM n=41,386) of diabetes

in UK patients aged 10 to 79 years old. Prevalence increased from 2.8% in 1996 to 4.3% in 2005 while the incidence rose from 2.71 per 1,000 person years in 1996 to 4.42 per 1,000 person years in 2005. Possible mechanisms for this increase include an ageing population and rising obesity levels, as the proportion of individuals newly diagnosed with T2DM who were obese increased from 46% to 56% between 1996 and 2006 (3). Geiss *et al* (2006) looked at the factors associated with the incidence of Diabetes in the United States using the National Health Interview Survey data from 1997 to 2003 (4). This study also showed an increasing incidence, again of note here was its correlation with obesity. Their multivariate adjusted incidence increased with age and BMI, implying obesity is a major factor in the increasing incidence of T2DM (4). Table 1.1.2 is taken from the report 'Diabetes: State of the Nations 2006' and shows the estimated prevalence of diabetes in the whole of the UK in 2005 and 2006 (2).

| <u>Nation</u> | <u>Prevalence</u> | <u>Number of people</u> |
|------------------|-------------------|-------------------------|
| UK | 3.5% | 2,238,000 |
| England | 3.6% | 1,891,000 |
| Northern Ireland | 3.1% | 55,000 |
| Scotland | 3.4% | 170,00 |
| Wales | 4.1% | 127,000 |

Table 1.1.2. Prevalence of diabetes in the UK 2005/06 (2)

Diabetes is a huge burden on the NHS, accounting for about £1 billion in annual expenditure (about 5% of total expenditure) (2). Diabetes is the leading cause of blindness in the working age population (2, 5). One

thousand patients with diabetes start dialysis due to renal failure in the UK every year and about 20-25% patients starting renal replacement programmes are patients with diabetes (2, 5). In patients with T2DM the average life expectancy is reduced by eight to ten years and about 70% of deaths are attributable to atherosclerotic disease (5).

b. Diabetes and Cardiovascular Disease

It is well known that patients with T2DM are at an increased risk of cardiovascular (CV) disease which accounts for 70% of total mortality (6). In patients with T2DM insulin resistance results in impaired glucose metabolism along with increased likelihood of hypertension, central obesity, endothelial dysfunction, abnormal lipid profiles and prothrombotic factors (7, 8). Unfortunately not only is the incidence of these CV risk factors increased in patients with T2DM but T2DM is itself a major CV risk factor (9). There is a two to four fold risk of developing coronary heart disease (CHD), stroke and peripheral vascular disease (PVD) in T2DM than in patients without diabetes (6, 7). Patients with diabetes are more likely to develop multi-vessel coronary artery disease (CAD) which is itself at increased risk of plaque ulceration and thrombosis (10). Similar differences have been described in peripheral blood vessels (10). The mortality after cardiac events (including sudden death) is also significantly increased compared to the non-diabetic population (7, 11). Further more, despite death from CV disease falling over the last 35 years, there has not been a decline in CV deaths in T2DM (7). Atherosclerosis and its complications in T2DM are now well accepted as important target areas for

treatment and funding and there are national targets and guidelines to help address this problem. Increased CAD is not the only myocardial problem in diabetes e.g. left ventricular hypertrophy (LVH) is also common in those with diabetes and would appear to be at least as adverse as CAD. This is discussed in more detail in Section 2.

2. Left Ventricular Hypertrophy in Patients with Diabetes

a. Definitions of Left Ventricular Hypertrophy

LVH is a condition whereby the cardiac myocyte responds to biomechanical stress from either extrinsic or intrinsic sources. The precise mechanisms whereby LVH arises are discussed later. At a cellular level, the cardiomyocyte increases in size and has enhanced protein synthesis (12). There are three main ways to detect LVH clinically: electrocardiogram (ECG), echocardiogram (echo) and cardiac magnetic resonance imaging (CMR) scanning.

i. Electrocardiographic Criteria for Left Ventricular Hypertrophy

ECG criteria for LVH include the Sokolow Lyon or Cornell voltage criteria. The Sokolow Lyon criteria are defined as the sum of the S wave in V_1 and the R wave from the highest of either V_5 or V_6 . LVH is diagnosed if the sum is greater than 35mm. The Cornell voltage criterion is defined as the sum of the S wave in V_3 and the R wave in aVL. Values of greater than 20mm in women and 28mm in men are defined as representing LVH on an ECG. It is now well accepted the Sokolow Lyon and Cornell voltage criteria perform poorly at detecting LVH in comparison with echo (13, 14). When the ECG criteria that was used in the LIFE study were applied to a population of patients with diabetes, it was found many patients with echo LVH would be missed (15).

ii. Echocardiographic Criteria for Left Ventricular Hypertrophy

Echocardiographic measurements of LVH are generally defined using the American Society of Echocardiography (ASE) recommendations (16). It is recommended that M mode measurements are taken at end diastole at the onset of the QRS complex. Left ventricular mass (LVM) is indexed to body surface area (BSA) and LVH defined as greater than 95g/m^2 in women and 115g/m^2 . LVM can also be indexed to height to avoid underestimating the prevalence of LVH in obese patients. The ASE recommends cut offs as $44\text{g/m}^{2.7}$ for women and $48\text{g/m}^{2.7}$ for men when LVM is index to height. Both these methods of assessing LVH have been prognostically validated (16).

iii. Cardiac MRI Criteria for Left Ventricular Hypertrophy

CMR scanning of the heart is an accurate measurement of LVM and is highly reproducible (17-19). There is a poor correlation between echo calculated LVM and CMR calculated LVM. In general echo M mode LVM calculations show overestimation and large measurement variability when compared with CMR measurements (20). Simpson *et al* (2009) found that mean LVM was higher in their echo derived data than the mean CMR derived LVM index (LVMI) in their study of 51 subjects (21). This is a common finding that has been previously reported and is due to the ways by which LVM is estimated (22). The echo method makes large geometric assumptions because it is based on two-dimensional data, whereas the CMR method makes fewer geometric assumptions because it is calculated from three-dimensional data.

There are limitations however when using CMR to measure LVM. There is inter-observer variability when deciding which basal and apical slices to include and each slice has a large cross sectional area which can have large effects on mass and volume measurements (23). There are also difficulties encountered when defining the most basal slice adjacent to the mitral valve plane due to partial volume effects (23). The values that are considered as a normal range for LVM using CMR are therefore lower than for echo.

The normal ranges for LVM derived from CMR vary depending on whether fast gradient echo imaging techniques (FLASH) or steady state free precession (SSFP) techniques are used. The traditional FLASH images are accurate for assessment of assessment of LV volumes, mass and function (24). The SSFP images are quicker but have greater contrast at the epicardial border with less blood flow dependence and greater fat to myocardial contrast at the epicardial border (24). Therefore there are differences in CMR derived LVM when using different imaging techniques. Malayeri *et al* (2008) analysed the results from 50 individuals using the two techniques, FLASH and SSFP (24). They found LVM was significantly lower using the SSFP technique, with a mean difference of 4.8g at end systole and 6.0g at end diastole.

Average LVM values using SSFP techniques: Clay *et al* (2005) studied 40 normal subjects, comprising 20 males and 20 females aged between 19 to 54 (23). They used 1.5 Tesla (T) MRI machine and also used SSFP sequences. Alfakih *et al* (2003) analysed their cohort of 60 normal subjects with SSFP

sequences as well as FLASH sequences (25). Table 1.2.1 shows the normal values derived from these two studies using SSFP techniques.

| | Men | | Women | |
|--|--------------------|---------------------|--------------------|---------------------|
| Parameter | Mean +/- SD | Normal range | Mean +/- SD | Normal range |
| LVM (g) (Clay et al) | 136.2 ± 20.5 | 95-177 | 69.2 ± 15.5 | 65-127 |
| LVM (g) (Alfakih et al) | 128.8 ± 23.2 | 85-181 | 88.1 ± 11.7 | 66-114 |
| LVMi (g/m²) (Clay et al) | 68.0 ± 8.8 | 50-86 | 53.7 ± 8.9 | 36-72 |
| LVMi (g/m²) (Alfakih et al) | 62.4 ± 7.6 | 46-83 | 48.8 ± 5.4 | 37-67 |
| LVM indexed to height (g/m) (Clay et al) | 76.0 ± 10.4 | 55-89 | 54.9 ± 15.0 | 25-85 |
| LVM indexed to height (g/m) (Alfakih et al) | 73.5 ± 11.9 | 51-100 | 54.2 ± 6.8 | 41-69 |

Table 1.2.1. The normal values for LVM and LVM indexed to body surface area and height using SSFP MRI (23, 25).

Average LVM values using FLASH imaging techniques: Natori *et al* (2006) performed CMR on 400 men and 400 women of different races at random (26). They excluded patients with traditional CV risk factors. The female group comprised of 170 white, 80 African-American, 83 Hispanic and 67 Asian-American volunteers. The male group included 68 white, 98 African-American, 78 Hispanic and 56 Asian-American volunteers. The MRI machine

was a 1.5T and the imaging technique was FLASH. In comparison with Natori *et al* (2006), Alfakih *et al* (2003) studied 60 normal subjects, 30 male and 30 female aged 20-65 and produced normal values for FLASH techniques (25). Table 1.2.2 shows the normal values for LVM and LVM indexed to body surface area and height for both studies.

| | Men | | Women | |
|---|--------------------|---------------------|--------------------|---------------------|
| Parameter | Mean +/- SD | Normal range | Mean +/- SD | Normal range |
| LV mass (g) (Natori et al) | 163.8 ± 35.8 | | 113.6 ± 24.2 | |
| LV mass (g) (Alfakih et al) | 154.2 ± 26.7 | 108-211 | 103.4 ± 13.5 | 82-132 |
| LV mass indexed to body surface area (g/m²) | 85.1 ± 15.2 | | 66.9 ± 10.9 | |
| LV mass indexed to body surface area (g/m²) (Alfakih et al) | 75.1 ± 8.8 | 60-96 | 57.3 ± 5.6 | 4 -77 |
| LV mass indexed to height (g/m) (Natori et al) | 94 ± 19 | | 71 ± 14 | |
| LV mass indexed to height (g/m) (Alfakih et al) | 87.9 ± 13.4 | 65-115 | 63.6 ± 7.8 | 50-80 |
| LV mass indexed to body mass index (g/kg/m²) (Natori et al) | 6.32 ± 1.21 | | 4.39 ± 0.82 | |

Table 1.2.2. The normal values for LVM and LVM indexed to body surface area, height and body mass index using FLASH MRI (25, 26).

Cain *et al* (2009) more recently looked at 96 healthy volunteers aged 11-81 years with 50 of the subjects being male (27). They used a 1.5T machine and

FLASH MRI and found that LVM varied over a broad age range as LVM rises in adolescence and peaks in middle age. Table 1.2.3 shows a summary of the predicted lower, mean and upper limits for normal LV parameters in males and females of different ages.

| Age (y) | LVM (g) | | | LVMBSA (g/m ²) | | | EDV (ml) | | | EDVBSA (ml/m ²) | | | ESV (ml) | | | ESVBSA (ml/m ²) | | | SV (ml) | | | SVBSA (ml/m ²) | | | EF (%) | | |
|----------------|---------|------|-------|----------------------------|------|-------|----------|------|-------|-----------------------------|------|-------|----------|------|-------|-----------------------------|------|-------|---------|------|-------|----------------------------|------|-------|--------|------|-------|
| | Lower | Mean | Upper | Lower | Mean | Upper | Lower | Mean | Upper | Lower | Mean | Upper | Lower | Mean | Upper | Lower | Mean | Upper | Lower | Mean | Upper | Lower | Mean | Upper | Lower | Mean | Upper |
| MALES | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 11-20 | 108 | 152 | 197 | 64 | 87 | 110 | 84 | 138 | 192 | 53 | 78 | 104 | 12 | 45 | 79 | 9 | 26 | 42 | 58 | 94 | 129 | 35 | 52 | 70 | 52 | 67 | 82 |
| 21-30 | 150 | 193 | 235 | 73 | 95 | 118 | 115 | 167 | 219 | 58 | 82 | 107 | 32 | 64 | 96 | 16 | 32 | 48 | 68 | 102 | 137 | 33 | 51 | 68 | 51 | 66 | 81 |
| 31-40 | 154 | 196 | 238 | 74 | 96 | 119 | 113 | 165 | 217 | 57 | 81 | 105 | 35 | 67 | 99 | 17 | 33 | 49 | 64 | 98 | 132 | 31 | 48 | 65 | 51 | 65 | 80 |
| 41-50 | 149 | 191 | 233 | 73 | 95 | 117 | 105 | 156 | 208 | 53 | 77 | 102 | 33 | 65 | 97 | 16 | 32 | 48 | 57 | 91 | 125 | 29 | 46 | 63 | 50 | 65 | 79 |
| 51-60 | 141 | 183 | 225 | 70 | 92 | 114 | 94 | 145 | 197 | 48 | 73 | 97 | 29 | 61 | 93 | 14 | 30 | 46 | 51 | 85 | 119 | 26 | 43 | 60 | 50 | 64 | 79 |
| 61-70 | 130 | 173 | 216 | 66 | 89 | 111 | 80 | 133 | 185 | 43 | 67 | 92 | 23 | 55 | 88 | 12 | 28 | 44 | 43 | 78 | 112 | 22 | 39 | 57 | 49 | 64 | 78 |
| 71-80 | 118 | 163 | 207 | 61 | 85 | 108 | 65 | 120 | 174 | 36 | 62 | 88 | 15 | 49 | 83 | 8 | 26 | 43 | 35 | 71 | 106 | 16 | 36 | 55 | 49 | 63 | 78 |
| FEMALES | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 11-20 | 80 | 125 | 171 | 56 | 79 | 101 | 77 | 120 | 162 | 55 | 76 | 96 | 13 | 37 | 60 | 10 | 22 | 34 | 53 | 84 | 116 | 36 | 53 | 71 | 55 | 71 | 86 |
| 21-30 | 98 | 142 | 186 | 60 | 82 | 104 | 76 | 119 | 161 | 52 | 72 | 93 | 17 | 40 | 63 | 12 | 24 | 35 | 49 | 81 | 112 | 32 | 49 | 66 | 55 | 70 | 86 |
| 31-40 | 98 | 142 | 186 | 59 | 81 | 103 | 75 | 118 | 160 | 49 | 70 | 90 | 19 | 42 | 64 | 12 | 24 | 36 | 46 | 77 | 108 | 29 | 46 | 63 | 54 | 70 | 85 |
| 41-50 | 96 | 140 | 183 | 58 | 80 | 102 | 74 | 116 | 158 | 47 | 68 | 88 | 20 | 43 | 65 | 13 | 25 | 36 | 43 | 74 | 105 | 26 | 43 | 60 | 54 | 69 | 85 |
| 51-60 | 93 | 136 | 180 | 57 | 79 | 101 | 73 | 115 | 158 | 46 | 66 | 87 | 21 | 44 | 67 | 13 | 25 | 37 | 40 | 71 | 103 | 24 | 41 | 58 | 53 | 69 | 84 |
| 61-70 | 88 | 133 | 177 | 55 | 77 | 100 | 72 | 114 | 157 | 45 | 66 | 86 | 22 | 45 | 68 | 14 | 26 | 38 | 37 | 69 | 100 | 23 | 40 | 57 | 53 | 69 | 84 |
| 71-80 | 83 | 129 | 174 | 53 | 76 | 99 | 69 | 113 | 158 | 44 | 65 | 87 | 22 | 46 | 70 | 14 | 26 | 39 | 34 | 66 | 99 | 21 | 39 | 57 | 53 | 68 | 83 |

BSA = body surface area, EDV = end diastolic volume, EF = ejection fraction,
ESV = end systolic volume, LVM = left ventricular mass, SV = stroke volume.

Table 1.2.3. Summary of the predicted lower, mean and upper limits for normal LV parameters in males and females of different ages (27).

Recently, newer 3T MRI machines are being phased in and the above data was all derived on 1.5T machines. Both sequences have been shown to have no difference in the quantification of LVM and volumes when using either 1.5T or the higher field strength 3T MRI machines and therefore normal LVM ranges should be transferable to both types of MRI machines (28).

b. Prevalence of Left Ventricular Hypertrophy

LVH is common in a number of conditions namely CAD, PVD, hypertension and obesity. The Framingham heart study found that prevalence of LVH increases with age as 33% of men and 49% of women aged 70 or older had LVH in their cohort (29). Framingham also found that a significant association between blood pressure (BP) and LVH was present even at levels of systolic pressure below 140 mm Hg (age adjusted). There is a nine fold (women) to tenfold (men) increase in age adjusted LVH prevalence from the leanest to the most obese group. In Framingham's multivariate analysis, age, BP, obesity, valve disease, and myocardial infarction were all independently associated with LVH in both sexes. Ang *et al* (2007) found the prevalence of LVH in CAD was as high as 73%, and interestingly 62% of these patients had non-hypertensive 24hour BP recordings (30). Up to half of patients with newly diagnosed PVD have LVH (31).

It is becoming increasingly recognised that diabetes is an independent predictor of LVH. Dawson *et al* (2005) performed an echo on 500 patients with T2DM to look for abnormalities of LV function (32). Standard echo M

mode measurements were taken of the LV and indexed to body surface area and height. LVH was highly prevalent, of the 371 patients in whom LVM could be successfully measured, 264 (71%) had LVH when LVM was indexed to height and 159 (43%) when LV mass was indexed to body surface area. When this data was published these measurements were applied to the old higher ASE guidelines for defining echo LVH as LVMI greater than 110 g/m^2 in women and greater than 134 g/m^2 in men and LVMI to height as greater than $47 \text{ g/m}^{2.7}$ in women and greater than $50 \text{ g/m}^{2.7}$ in men. When the new ASE definitions of LVH (LVMI greater than 95 g/m^2 in women and greater than 115 g/m^2 in men and LVMI to height as greater than $45 \text{ g/m}^{2.7}$ in women and greater than $49 \text{ g/m}^{2.7}$ in men) were applied to their data they found the prevalence of LVH was even higher and is summarised in table 1.2.4.

| | Mild LVH (%) | Moderate LVH (%) | Severe LVH (%) | Overall prevalence (%) |
|---------------------------|--------------|------------------|----------------|------------------------|
| Indexed to BSA: | | | | |
| Women | 18 | 21 | 26 | 65 |
| Men | 18 | 15 | 30 | 63 |
| Indexed to height: | | | | |
| Women | 19 | 19 | 45 | 85 |
| Men | 15 | 16 | 41 | 72 |

Table 1.2.4. Prevalence of LVH as defined by the new ASE guidelines (32).

Nakamura *et al* (1994) showed in a smaller number of patients, again using echo M mode, that LVMI in hypertensive patients with glucose intolerance

was significantly higher than that in hypertensive patients without glucose intolerance. The mean LVM in the glucose intolerance and hypertension group was $115.6 \pm 28.2 \text{ g/m}^2$ whereas the mean LVM in the hypertensive only group was $102.1 \pm 22.1 \text{ g/m}^2$ ($P < 0.05$) (33). Framingham data showed that after accounting for confounding factors, in women with diabetes there is an independent association of higher LVM and wall thickness (34). A survey performed on patients attending our local diabetes clinic found LVH was present in 32% (57 of 173) of patients with T2DM, independent of BP or the use of angiotensin-converting enzyme (ACE) inhibitors (35).

Barrios *et al* (2009) used ECG criteria to look at the prevalence of LVH in diabetic patients (36). LVH was more common again than in the patients without diabetes population at baseline. 37.5% of diabetic and 26.4% of non-diabetic patients fulfilled criteria of ECG LVH by the Cornell criteria ($p=0.02$), 25.7% and 23.2%, respectively, by Sokolow Lyon criteria ($p=0.18$), 11.8% and 13.7% by Cornell voltage index ($p=0.16$), and 14.3% and 11.6% by Sokolow Lyon voltage index ($p=0.10$). The differences in prevalence are different if echo or ECG criteria are used as it is well accepted the classic Sokolow Lyon and Cornell voltage criteria underestimate LVH when compared with echo, as discussed in the previous chapter (13, 14).

c. Increased Cardiovascular Risk Associated with Left Ventricular Hypertrophy

The presence of LVH on ECG or echo is well known to confer a higher risk of CV events and mortality from many studies over the last 20 to 30 years (37, 38). Levy *et al* (1990) studied 3,220 patients in the Framingham Heart Study to look at the relationship of echo LVH to the incidence of CV events (37). The patients included were over 40 years old and did not have apparent CV disease. They found that LVH predicts a higher incidence of clinical events attributable to CV disease, including death, and this was evident even after correcting for traditional risk factors. In men, the risk factor-adjusted relative risk of CV disease was 1.49 for each increment of 50g/m² in LVM corrected for height (95% CI, 1.20 to 1.85); in women, it was 1.57 (95% CI, 1.20 to 2.04).

Liao *et al* (1995) directly compared the survival of echo LVH with CAD. They included 1,089 black patients and followed them up for a mean of five years. Interestingly they found that LVH was associated with higher risk ratio than CAD (relative risk for LVH 2.4 (95% CI 1.7 to 3.2) and relative risk for multi-vessel CAD 1.6 (95% CI, 1.1 to 2.2)) (39). It is debatable whether this extra risk extends to Caucasian populations, as it is well known black patients have higher CV deaths and heart failure (40-43). Surprisingly this type of study has not been replicated on a wider scale in other race groups.

Vakili *et al* (2000) analysed the results of 45,545 patients published in 20 studies between 1960 and 2000 to review the association between LVH and CV events (38). The studies included in this large meta-analysis diagnosed LVH either on ECG, echo or on both. When ECG criteria for LVH was studied, LVH was associated with 1.6 to 4.0 fold higher risk of future CV events and included 14, 450 patients (38). The studies included varied on the criteria used to diagnose LVH on ECG. Therefore some patients may have met the criteria for one study but not the other. Studies using echo LVH included less patients, (n=3, 651) and had an adjusted relative risk (RR) of CV events of 1.5 to 3.5, see Figure 1.2.1 (38). Overall the events defining CV end points varied amongst the different studies and this should be borne in mind when reviewing the data, but regardless of this or the LVH definitions used, a strong trend was shown between LVH and adverse events in this meta-analysis (38).

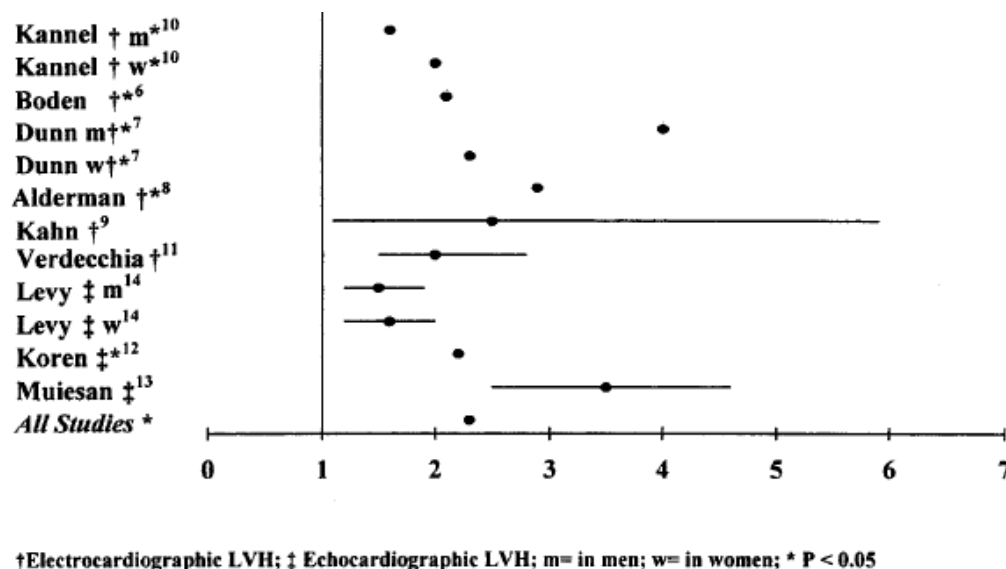


Figure 1.2.1 Summary of mean risk ratio for available studies of baseline LVH for CV events (38).

It is not clear whether men or women have worse outcome if they have LVH, and this is reflected in a number of studies. Levy *et al* (1990) used echo to diagnose LVH and found that the risk ratio for CV events was 2.0 (95% CI, 1.44 to 2.81) for women and 1.6 (95% CI, 1.14 to 1.94) for men. Dunn *et al* (1990) found the opposite. They studied 3,780 patients, using ECG to diagnose LVH, who attended Glasgow BP Clinic between 1968 and 1983. All-cause age-adjusted mortality (deaths per 1000 patient-years) was 41.4 for men and 22.1 for women (44).

d. Left Ventricular Hypertrophy and its Association with Increased Cardiovascular Risk

i. Myocardial Ischaemia and Left Ventricular Hypertrophy

In patients with LVH and normal coronary arteries, coronary flow reserve is reduced, which can lead to exertional myocardial ischaemia due to an inability to meet the metabolic needs of the thickened myocardium. Andren *et al* (1999) performed exercise tests on elderly male patients with either LVH and hypertension, or LVH and normal BP and compared these with healthy controls (n=58). None of the recruited patients had known CAD (45). The hypertensive subjects with LVH showed more pronounced ST depression on exercise testing when compared with the healthy group without LVH. In both of the LVH groups, more than 20% of the subjects had ST-segment depression greater than or equal to one millimetre (usually considered to be a significant change), compared with only 5% of the healthy group without LVH.

There are differing theories as to why coronary flow reserve is reduced in LVH. One possible mechanism is the result of increases in extra vascular compressive forces caused by LVH. This may result in wall stress leading to interstitial fibrosis and changes in the of coronary arteries (and small micro-arteries) leading to reduced myocardial blood flow (46). This hypothesis fits in with the observation that the degree of LVH correlates in a linear fashion with the amount of reduction in coronary flow reserve. Opherk *et al* (1984) first showed this by measuring coronary blood flow using the argon method in 12 control subjects and in 16 patients with hypertension and LVH at rest and after intravenous administration of dipyridamole. In the patients with hypertension and LVH, coronary blood flow response to dipyridamole was markedly reduced and during coronary vasodilatation there was a linear correlation between coronary resistance (46). Hamasaki *et al* (2000) confirmed similar findings, using a different technique of intravascular ultrasound, in the left anterior descending coronary artery (47). They studied 111 patients with normal coronary arteries divided into three groups: normal BP, hypertension and LVH, or hypertension and no LVH. The response to both acetylcholine and adenosine was significantly impaired in patients with LVH.

In contrast to the above studies, other researchers have found that impaired coronary flow reserve in hypertensive patients is not always related the degree of LVH. Gimelli *et al* (1998) has proposed the possible reason for these alternative findings is that endothelial dysfunction is caused by vascular remodelling such as media thickening, peri-vascular fibrosis or functional

vascular alterations independent of the degree of LVH (48). This may result in changes in the ability of the coronary arteries to vasodilate. This was first shown by Vogt *et al* (1992), they studied over 200 hypertensive patients with no CAD. They found that LVM and coronary vascular resistance were not directly related (49). Gimelli *et al* (1998) studied 50 untreated hypertensive patients and 13 normotensive patients and measured coronary reserve and resistance before and after dipyridamole (48). They found that LVM was not correlated with global myocardial blood flow. Patients with LVH did have a heterogeneous flow pattern with regional defects and normal blood flow in non-affected areas of the myocardium.

With regard to coronary flow reserve in patients with diabetes, a Japanese group looked at 92 subjects consisting of 70 diabetic patients without overt cardiac disease and 22 normal controls to assess myocardial blood flow reserve during exercise using myocardial contrast echocardiography (50). Myocardial blood flow reserve was significantly reduced in patients with diabetes compared with controls. They showed that diabetic patients have exercise induced delayed onset of LV relaxation in association with impaired coronary microcirculatory function in the absence of co-existent heart disease.

In summary it may be both mechanisms for myocardial ischaemia co-exist to different degrees in patients with LVH and hypertension. Because there is also evidence that there are changes in coronary microvasculature specifically in diabetic patients, the combination of hypertension, diabetes and LVH will

have a significant impact on coronary artery flow reserve and hence ischaemia.

ii. Arrhythmias and Left Ventricular Hypertrophy

For a long time it has been accepted that LVH is associated with ventricular ectopy, ventricular arrhythmias and cardiac sudden death. Messerli *et al* (1984) looked at a small number of patients with LVH and found they had more premature ventricular ectopics (51). McLenachan *et al* (1987) also showed that not only are ventricular ectopics common in patients with LVH, but so were episodes of non-sustained ventricular tachycardia (VT) (52). 16% of their patients with LVH had non-sustained VT of five beats or more. Siegel *et al* (1990) found the odds ratio (OR) for having episodes of VT in LVH was 2.3 (confidence interval of 0.7 – 7.1) in their cohort of patients (53). These studies suggest non-sustained VT is highly prevalent in patients with LVH but none of these studies actively excluded CAD and it is known there is also a strong association with CAD. Ghali *et al* (1991) was the first to show the association of LVH and ventricular arrhythmias in patients with normal coronary arteries proven by coronary angiography (54). In their cohort, they showed the frequency and complexity of ventricular arrhythmias was significantly related to the presence of LVH. They found for every one millimetre increase in the thickness of inter-ventricular septum or posterior wall there was an associated two to threefold increase, respectively, in the occurrence and complexity of ventricular arrhythmias (54). The association

between LVH and ventricular arrhythmias has also been confirmed by electrophysiological testing (55).

The reasons why patients with LVH may be prone to ventricular arrhythmias are due to changes in the myocardium in LVH. Cosin Aguilar *et al* (1993) explains that LVH leads to electrical changes, growth of the collagen matrix and ischaemia that contribute to arrhythmias (56). These myocardial changes increase re-entry electrical mechanisms, long QTc and prolonged action potentials further contributing to the risk of ventricular arrhythmias (47).

iii. Left Atrial Size and Left Ventricular Hypertrophy

Another mechanism whereby LVH increases CV risk is its association with left atrial (LA) dilatation. LVH leads to reduced compliance of the LV and leading to the LA needing to provide higher filling pressures leading to diastolic heart failure and eventually to LA dilatation (57, 58). LA dilatation is related to LV mass and it does not matter if the LVH is eccentric or concentric (59). Other features strongly associated with increased LA size in LVH include high BMI, high systolic BP and the presence of mitral regurgitation and atrial fibrillation (AF) (60). Stritzke *et al* (2009) studied the association of obesity and hypertension to LA volume (61). Patients were followed up prospectively over 10 years. After adjustment for age and sex, the OR between obesity and LA enlargement was 2.7 while hypertension was less at 1.1. It would therefore seem obesity is a greater determinant of LA enlargement than hypertension.

LA size is important because it is considered a CV risk factor in its own right. The LIFE trial looked at LA diameter by annual echo correlated with CV events in 881 hypertensive patients (41% women) with ECG LVH during a mean follow up period of 4.8 years (62). They found that baseline LA diameter predicted the incidence of CV events (defined as death, myocardial infarction or stroke) with a hazard ratio of 1.98 (95% confidence interval [CI] 1.02 - 3.83) adjusted for significant effects of Framingham risk score and history of AF. Framingham also showed a correlation between LA size, death and stroke (63). All subjects 50 years of age and older from the Framingham Heart Study were studied with an 8 year of follow up. After multivariable adjustment, for every 10-millimetre increase in LA size, the relative risk of stroke was 2.4 in men (95% confidence interval 1.6 - 3.7) and 1.4 in women (95% confidence interval 0.9 - 2.1); the relative risk of death was 1.3 in men (95% confidence interval 1.0 - 1.5) and 1.4 in women (95% confidence interval 1.1 to 1.7). LA size has also been associated with sudden cardiac death in patients with heart failure (64).

LA dilatation (which we now know is related to LVH) and diabetes are risk factors for development of AF, therefore special consideration to AF in diabetes is given below.

iv. Atrial Fibrillation in Diabetes

Why is AF important in diabetes and LVH? It is becoming increasingly recognised that in addition to traditional risk factors for AF, such as

hypertension, ischaemic (IHD) and heart failure, diabetes is also a risk factor for AF. The exact pathophysiology for this observation is unclear but is possibly related to the dilated LA found in patients with LVH and also the direct glucose affects on the myocardium causing electrical instability. Obesity and hypertension are also strongly associated with LA dilatation, which in turn is a strong risk factor for AF, as discussed previously. Therefore co-morbid vascular disease associated with diabetes is one possible reason for the association of diabetes with AF. Rutter *et al* (2003) investigated the impact of insulin resistance on heart structure and function in a community based sample of Framingham study patients (65). They found that the more severe glucose intolerance, the higher the LA size in both men and women. Lind *et al* (1996) also found a correlation between diabetes and LA size (66).

AF in diabetes is important because diabetes may mask the cardiac symptoms associated with the onset of AF and this masking of symptoms is a potential mechanism for the increased risk of stroke in patients with diabetes, i.e. if AF is under diagnosed then anti-coagulation will not be considered. The development of AF in a diabetic patient has also been shown to increase the risk of stroke in diabetic patients (67). Stroke risk is most commonly measured by the well-validated 'CHADS2' score (68, 69). One large study, Wilhelmsen *et al* (2001), did not find a correlation between AF and diabetes (70), but subsequent larger studies have shown significant correlations and these are summarised in table 2.5.

| Author | Total number of patients | Prevalence of AF in diabetics | Prevalence of AF in non diabetics | Odds ratio | 95% Confidence interval | Hazard ratio | 95% Confidence interval | p value |
|-----------------------|--------------------------|-------------------------------|-----------------------------------|-----------------------|-------------------------|--------------|-------------------------|---------|
| Movahed et al (2005) | 845748 | | | 2.13 | 2.10 - 2.16 | | | <0.05 |
| Östgren et al (2004) | 1739 | 4 | 2 | 2 | 0.9 - 4.7 | | | <0.05 |
| Nichols et al (2009) | 17372 | 3.6 | 2.5 | | | 1.26 | 1.08 - 1.46 | <0.05 |
| Benjamin et al (1994) | 4731 | | | 1.4 (men) 1.6 (women) | | | | <0.05 |
| Aksnes et al (2008) | 15245 | | | | | 1.49 | 1.14 - 1.94 | <0.05 |
| Psaty et al (1997) | 5201 | | | 1.08 | 1.03 - 1.13 | | | <0.05 |

Table 1.2.5 Comparison of the association of AF with Diabetes from the major published studies expressed as odds and hazard ratios.

In an analysis of all 84,5748 in-patient records from all the Veterans Health Administrations hospitals in America (71), Movahed *et al* (2005) reported a significant correlation between diabetes and AF. AF occurred in 14.9% of diabetic patients compared with 10.3% in the control group of patients with hypertension but no diabetes. Atrial flutter occurred in 4% of diabetic patients compared with 2.5% in the control group. When congestive heart failure, CAD and LVH were accounted for, diabetes still remained a strong independent risk factor for AF and atrial flutter, with odds ratios of 2.13 and 2.20 respectively. This study is by far the largest study looking at the correlation between diabetes and AF. There are a number of drawbacks in that this study was retrospective and was conducted in hospitalised patients only.

The two other larger studies, Nichols *et al* (2009) and Aksnes *et al* (2008) reported similar findings from large diabetes registries (72, 73). Nichols *et al* (2009) found after adjustment for other risk factors, diabetes was associated with a 26% increased risk of AF among women, but intriguingly diabetes was not a predictor among men. The Framingham Heart Study, Östgren *et al*

(2004) and Psaty *et al* (1997) looked at smaller numbers of patients but found similar significant correlations (74-76). Aksnes *et al* (2008) studied large numbers of patients in the VALUE trial to see who developed AF (73). Of the patients who developed AF during the follow up period, diabetic patients had a significantly higher chance of developing AF that was also more persistent.

v. Summary

The development of LVH in a diabetic patient is an ominous sign. This is due to not only the already increased CV risk that comes with being diabetic but also because LVH has been shown to be a serious risk factor in its own right in a number of ways. LVH, hypertension and diabetes affects coronary flow reserve inducing ischaemia. LVH induces arrhythmias such as VT and AF that are associated with considerable morbidity and mortality. LVH impedes ventricular filling and leads to diastolic heart failure that may even later transform into systolic heart failure. Finally, LVH increases LA size, which is a CV risk factor in its own right. Therefore the combination of these factors in a diabetic patient with LVH means it should be an important target for future therapies.

3. **The Development of Left Ventricular Hypertrophy**

Hypertension is the most commonly thought of and studied cause of LVH due to its haemodynamic effects on the LV. However BP trials have suggested that different agents regress LVH more than others independent of BP, i.e. ACE inhibitors and Angiotensin Receptor Blockers (ARBs) regress LVH more than agents such as beta-blockers, and the degree of LVH varies even for similar levels of hypertension (77-79). These trials are discussed in detail in chapter 4: 'Treating Left Ventricular Hypertrophy'. It seems there must be more to LVH than BP and the view that BP is the only pathogenic mechanism in the development of LVH is now considered too simplistic. Here, pathogenic theories for the development of LVH are discussed individually.

a. **Blood Pressure and Obesity**

The main theory for the development of LVH is based around changes in the myocardium in response to haemodynamic burden, most commonly due to increase after-load as a result of hypertension. Increase in haemodynamic over load can be either due to increased pressure (e.g. hypertension, aortic stenosis) or volume overloads (e.g. aortic regurgitation, mitral regurgitation or anaemia). The response of the heart to haemodynamic pressure increases causes the heart to compensate in three ways: shifting of the Frank-Starling curve to the right leading to increased cardiac output, increase in cardiac muscle mass to adjust to increased after-load and neuro-hormonal mechanisms to increase contractility (80). It is the increase in muscle mass

as a result of myocardial hypertrophy (not hyperplasia) that is the key long-term compensatory mechanism in response to a haemodynamic burden. Pressure overload tends to lead to concentric hypertrophy and volume overload tends to lead to eccentric hypertrophy.

Thus hypertension is a key cause for the development of LVH in the general population and there is a correlation between the degree of hypertension and the development of LVH (29, 81, 82). In particular, ambulatory BP (ABPM) monitoring is superior to office BP monitoring of hypertension. Verdecchia *et al* (1990) showed in 165 untreated hypertensive patients compared with 92 healthy patients that 24 hour ABPM was better at predicting LVH than office BP readings (83). There was a stronger correlation between ABPM than office BP in terms of predicting LVH. They also correlated ABPM to office BP and when ABPM was higher than predicted, this group had the highest prevalence of LVH (6-10% and 35-39% respectively) (84, 85).

We already know LVH is common in people with diabetes. Hypertension and obesity are more common in patients with T2DM; in fact T2DM patients are up to twice as likely to develop hypertension as without diabetes. Obesity is independently associated with LVH, and the combination of hypertension and obesity is additive (17 fold increase in risk of developing LVH) (82, 86, 87). LVH may be more common in diabetes because of the close relationship between insulin resistance, obesity and hypertension (88-90). However, patients of normal weight and BP are at risk of LVH. Dawson *et al* (2005) found that LVH occurred independently of BP in a cohort of 500 diabetic

patients (32). There were more patients in the LVH group who had a history of hypertension (8% with no LVH and 13% with LVH) and this was significant for only one LVM parameter. Framingham found that 28% of females in their cohort aged over 60 years with BP below 139mmHg had LVH on echo (87).

There must be other factors contributing to the development of LVH that are not mediated just by obesity and/or BP, and it seems LVH is mediated by the interaction of other variables such as genes, oxidative stress (OS), endothelial function, renin angiotensin system (RAS) and direct effects of insulin and glucose on the myocardium. Of course, these factors also interplay with hypertension and obesity, and are discussed in turn below.

b. Oxidative stress in Diabetes

i. The Concept of Oxidative Stress

Reactive oxygen species (ROS) is a collective term for the by products of the metabolism of oxygen in a normal cell that can be also abnormally increased in particular diseases and stress states such as atherosclerosis, neuro-degeneration, renal disease and cancer. ROS include super oxide anions (O_2^-) and hydrogen peroxide (H_2O_2) that undergo further reactions to produce more ROS. In particular, O_2^- interacts with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$) and H_2O_2 is converted to hydroxyl radicals (OH^\cdot) via Fenton chemistry, as shown in Figure 1.3.1 (91, 92).

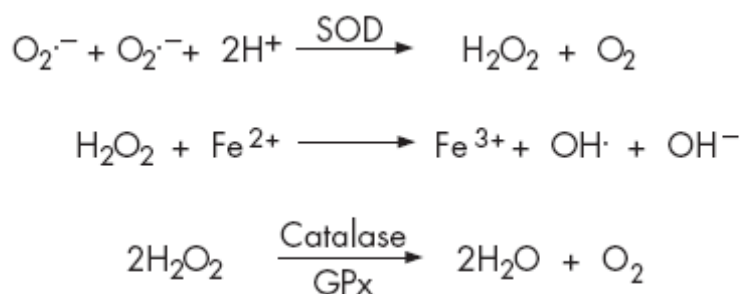


Figure 1.3.1. The principle reactions involved in the generation and degradation of hydrogen peroxide (92).

$\text{O}_2^{\cdot-}$ is formed by mitochondrial respiration or by various enzymes including NAD(P)H oxidase, NO synthase and xanthine oxidase (XO) (91). XO is the enzyme blocked by allopurinol, which is discussed in detail later. In normal cells, these ROS are maintained at low levels by two principle enzymes, superoxide dismutase (SOD) and catalase, that mop-up and scavenge ROS and degrade them to inactive molecules (92). SOD is found in three various forms, mnSOD, CuSOD and ZnSOD. In the human heart, it is estimated that mnSOD makes up 90% of the activity in myocytes, whereas CuSOD and ZnSOD is extracellular (91). In fact, homozygous mutant mice for the mnSOD gene die rapidly of cardiomyopathy (93). MnSOD is also implicated in myocardial adaptation after induced myocardial infarction in rats (94). The redox state of a cell describes the balance between generation and removal of ROS. ROS cause damage to cell signalling, proteins, DNA and RNA due to their unpaired valence shell electrons that can lead to a cycle of imbalance of the redox state which is known as OS (95). The regulation and chemistry involved in ROS is summarised in figure 1.3.2.

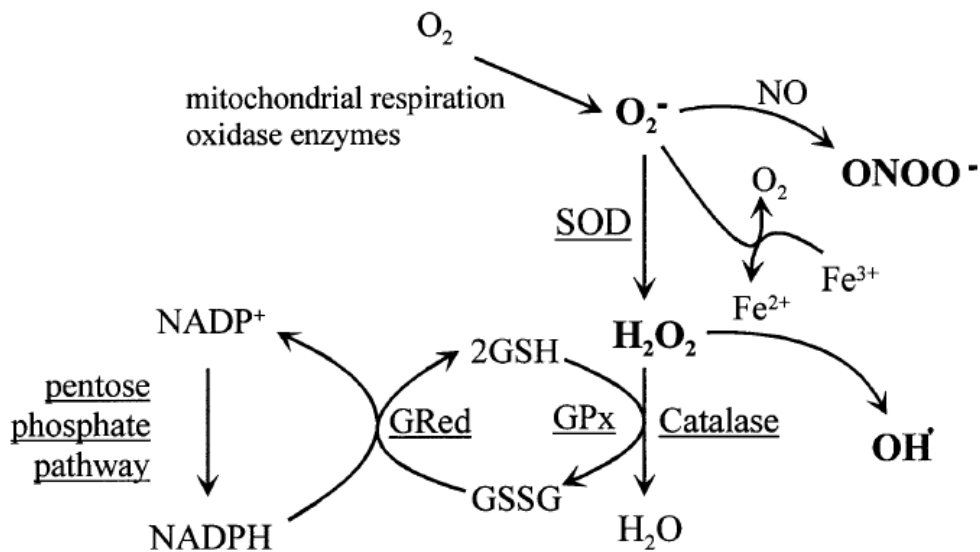


Figure 1.3.2. The basic pathway for the generation of ROS (95).

The two main sources of ROS in LVH and heart failure that have been implicated to date are XO and NADPH (92). Firstly, XO produces $O_2^{\cdot -}$ as a by-product of purine metabolism, (discussed in detail in 'section 5: Xanthine Oxidoreductase and Allopurinol') and XO related OS is found in pressure overload of the heart and heart failure, see Figure 1.3.3 (95). This has also been clearly demonstrated in several *in vitro* studies, including one in which XO was the source of the OS and caused the hypertrophic phenotype (96-98).



Figure 1.3.3. Role of XO in pressure overload and heart failure (95).

Secondly, NADPH oxidase catalyses the electron transfers from NADPH to oxygen, thereby producing O_2^- and NADPH oxidase activity that has been found to be increased in heart failure and LVH in experimental models (91, 93) (99-101).

ii. Oxidative Stress Causing Left Ventricular Hypertrophy

ROS are involved in the development and perpetuation of myocyte hypertrophy in two ways. Markers of increased OS, namely isoprostane-2, have been found systemically and in pericardial fluid in patients with heart failure (92, 98). In frank heart failure, ROS are thought to cause free radical oxidation and damage such as cell damage, apoptosis and necrosis (92). However, ROS can cause more subtle damage, as is the case in myocyte hypertrophy, where these ROS are thought to activate a variety of hypertrophy signalling kinases and transcription factors leading to the development and perpetuation of myocyte hypertrophy (95, 102). ROS also appear to mediate the hypertrophic response to other known hypertrophic stimuli in redox sensitive pathways, such as mechanical strain, angiotensin, tumour necrosis factor- α (TNF- α) and α -adrenergic, see Figure 1.3.4 (91, 95). A self perpetuating cycle appears to occur in that pressure overload produces OS which further exacerbates the hypertrophic response to the pressure overload (95).

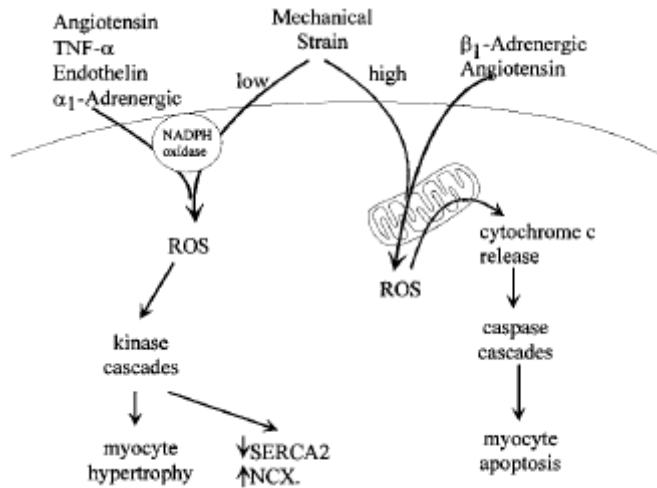


Figure 1.3.4. ROS mediated myocyte hypertrophy (91).

The main pathophysiological effects of OS in LVH and heart failure are summarised by Sneddon *et al* 2006, Figure 1.3.5. In summary, ROS cause damage in three principles ways. Oxidative damage causes myocyte dysfunction and cell death, inactivation of NO leads to endothelial dysfunction and deficiencies of redox signalling leads to hypertrophy and fibrosis.

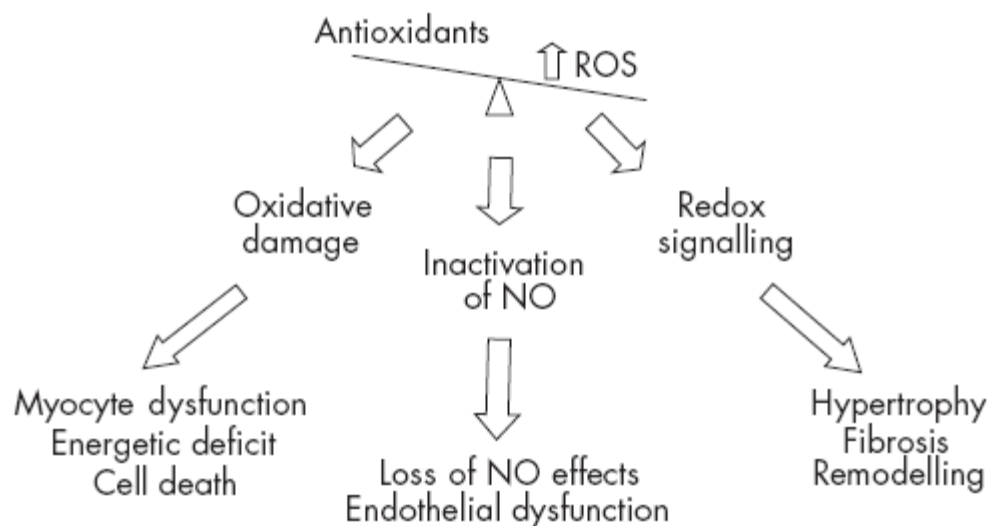


Figure 1.3.5. Pathophysiological effects of oxidative stress in LVH and heart failure (92).

iii. Diabetes as a Source of Oxidative Stress

Patients with diabetes are known to have increased OS. In T2DM there is principally increased mitochondrial ROS (especially O_2^-) production from free fatty acids in vessels and endocardium (103). Giacco *et al* (2010) have described a hyperglycaemic memory, whereby ROS generated due to hyperglycaemia in diabetes drive persistence pro-inflammatory genes that remain after glycaemia is normalised (103). These increased ROS may in part lead to the micro- and macro-vascular complications in diabetes, including LVH and heart failure.

The two main enzymes that are targets for treatments in reducing OS in heart failure and LVH are NADPH oxidase and XO. ACE inhibitors and ARBs inhibit ACE NADPH oxidase and are in widespread use (92). Inhibition of XO is the subject of this thesis and is discussed in detail.

c. Endothelial function

i. Principles of Endothelial Function

Until the 1980's the endothelium was thought of as an inert barrier in the blood vessel. In 1980, the landmark paper by Furchgott *et al* (1980), showed that the endothelium dilates in response to acetylcholine (104). Soon after, it was discovered NO also plays a vital role in the function of the endothelium (105).

Blood vessels are made up of three distinct layers, or tunicas, namely the intima, media and adventitia (106). The tunica intima is made up of a single layer endothelial cells which are flat epithelial cells, which have many functions that will be discussed in turn, and are attached to underlying connective tissue (106). The endothelium lines the intimal surface of the entire vascular tree, and is in fact the largest endocrine organ in the body. The endothelium has five main roles: it acts as a barrier between blood and tissues, it regulates haemostasis, regulates blood flow, regulates diapedesis (leucocyte extravasation) and is involved in cell signalling. This discussion will focus on the vasodilatory properties of the endothelium, as the other functions are out of the scope of this thesis.

Vasodilators

NO: NO was initially identified as Endothelial Derived Relaxing Factor (EDRF) in 1980, however EDRF was later found to be NO. NO is a potent vasodilator and its synthesis and actions are through the second messenger cyclic GMP that is calcium dependent. NO only acts locally as it has a half life of only a few seconds (107). NO is a free radical that reacts with other ROS, in particular superoxide to produce either the highly toxic peroxynitrite or the non-toxic nitrate (these reactions are described in more detail in the chapter on OS). NO is synthesised from the amino acid L-arginine in a variety of cells namely endothelial cells, platelets, neutrophils, monocytes, mast cells and adrenal cells (108). NO also plays a role in platelet aggregation, smooth muscle proliferation and endothelial cell inflammatory response. The precise mechanisms for the release of NO are still unknown, however shear stress is

probably the physiological stimulus for the continual release of NO, as NO activity is highest in arteries where shear stress is the highest (107). Synthesis of NO is also stimulated by acetylcholine, bradykinin and substance P and the release of NO accounts for the vasodilatory actions of these mediators, and hence are known as endothelium dependent vasodilators (107). Calcium stimulates nitric oxide synthase (NOS) to produce NO. NOS is found in three different forms: neuronal, macrophage and endothelial. Figure 1.3.6 shows the NO signalling pathway (109).

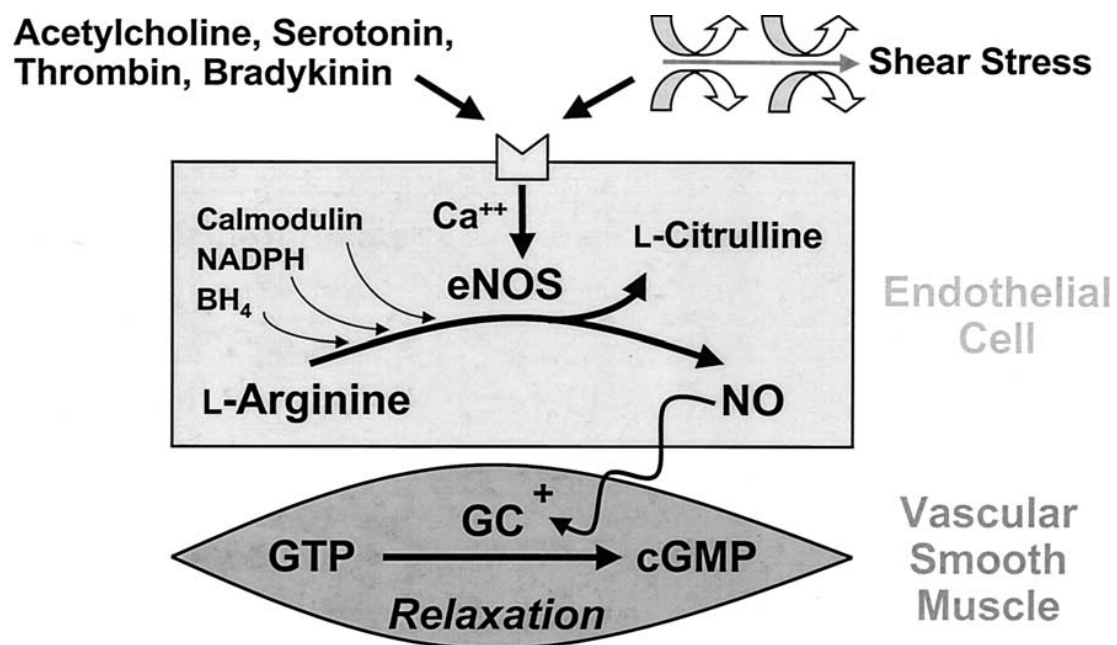


Figure 1.3.6. The nitric oxide signalling pathway (109).

NO is the dominant factor in vasodilatation, however there are other factors as discussed below. Removal of the endothelium usually leads to vasoconstriction and this effect is mimicked by NOS inhibitors (107).

Prostanoids: Prostacyclin and prostaglandin E₂ and D₂ are vasodilators (107). Blocking prostanoid synthesis with aspirin or non-steroidal anti-inflammatory drugs does not affect vascular tone, except in the kidneys (107).

Endothelial derived hyperpolarising factor (EDHF): EDHF was discovered by observations that vasodilatation was preserved in the presence of NOS and cyclo-oxygenase inhibitors (107). The role of EDHF is still largely unknown in human vasculature (107). It may be that EDHF plays an important role in small arteries.

Vasoconstrictors

The predominant influence on arteries is dilation, however, there are some important vasoconstrictor factors synthesised by the endothelium. These include endothelin, angiotensin II, various prostanoids and O₂⁻. Endothelin has a long duration of action and it is thought to provide background counteracting vasoconstrictor influence (110). The tissue RAS may contribute to vascular tone, as ACE is found in the endothelium. Endothelial derived angiotensin II can diffuse through the wall and stimulate neurones to produce noradrenaline, a possible link between the endothelium and nervous system (111). O₂⁻ probably influences vascular tone secondary to its inhibitory effects on NO mediated vasoconstriction, although it does have direct constrictor actions in some vessels (107).

ii. Endothelial Dysfunction in Diabetes

Endothelial dysfunction is the term used to describe the response of the endothelium to injury that leads to activation of signalling pathways and a cascade of inflammatory pathways (112). Deanfield *et al* (2007) argue that it should actually be called endothelial activation, as there is a switch from a quiescent phenotype towards a host defence response at the level of the endothelium (112). The understanding of endothelial dysfunction is tied in with knowledge of OS, as discussed previously. In principle, OS describes a state whereby there is reduced formation or accelerated degradation of NO, and this degradation of NO by ROS is key to the mechanisms underlying endothelial dysfunction (109).

eNOS uncoupling is a term that describes the fact that eNOS can switch to generate ROS under certain circumstances (113). The ROS that eNOS can switch to produce are O_2^- if the co-factor BH4 is oxidised or H_2O_2 if there is a deficiency of the substrate L-arginine (113). The normal function of eNOS requires, amongst other things, the essential cofactor (6R)-5,6,7,8-tetrahydro-L-biopterin (BH4) that is highly sensitive to oxidation by peroxynitrite. We have already discussed that O_2^- reacts with vascular NO to form the highly reactive peroxynitrite, and the cofactor BH4 is highly sensitive to oxidation by peroxynitrite. It is the diminished levels of BH4 that promote O_2^- production by eNOS.

In OS the usual NO mediated activation of cellular process changes because H_2O_2 that is produced by ROS and the uncoupling of eNOS can diffuse through cells in a similar fashion to NO (114). H_2O_2 reacts with cysteine groups in proteins to alter function that have differing results to NO, such as phosphorylation of transcription factors, nuclear chromatin remodelling, induction of transcription genes and protease activation (114).

It is thought that prolonged production of ROS may exceed the normal enzymic counterbalances and lead to abnormal endothelial activation (112). Obesity and T2DM in particular lead to increased glucose and free fatty acids and therefore there is more substrate available to the mitochondrion during oxidative phosphorylation leading to an imbalance and disruption of the redox state (112, 115). Importantly XO is an important source of ROS (112). Furthermore, CV risk factors such as hypertension, hypercholesterolemia, diabetes or chronic smoking stimulate the production of ROS in the vascular wall (113). NADPH oxidases represent major sources of this ROS and have been found to be 'up regulated' and activated in animal models of hypertension, diabetes, sedentary lifestyle and in patients with CV risk factors (113). The interaction of ROS with NO leads to a vicious cycle of endothelial activation and inflammation.

iii. Augmentation Index

The peripheral pulse and BP is the standard method of clinical assessment; however this does not necessarily reflect central aortic pressure. Central

aortic pressure is thought by some to be a better predictor of outcomes than simple peripheral BP (116). Applanation tonometry at the radial artery is an accurate, simple and reproducible method of measuring central pulse pressure, in particular an estimation of the ascending pressure wave can be made (116).

The central pressure waveform is a result of the summation of the forward transmission of the cardiac pressure (i.e. LV systolic pressure) impulse as it spreads through the vasculature and the backward reflection generated by the peripheral vascular system (117). Essentially, these points of reflection within the peripheral vascular system occur at the interface between large arteries and resistance vessels (peripheral arteries and arterioles) (116). Wave reflection results in augmentation of the aortic pressure wave, and is dependent on three factors: ventricular ejection contraction and length of contraction, amplitude of the reflected wave (reflecting the elasticity of the entire vascular tree) and velocity of the pulse wave from the peripheral tree (116, 117). A number of physical and physiological parameters affect the wave form, including systolic BP, heart rate and smaller stature (116). Augmentation index (Alx) is a measure of the effect of the wave reflection on the aortic pressure and it is calculated as the ratio of the increment in pressure due to early wave reflection, and thus gives an index of aortic stiffness, Figure 1.3.7.

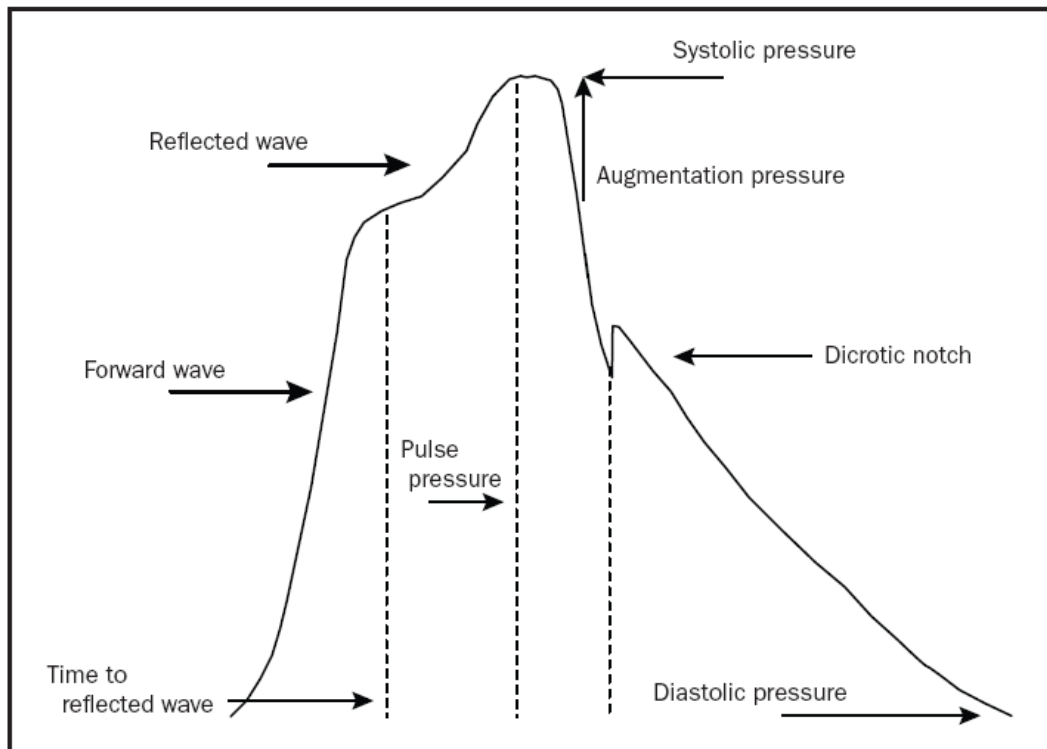


Figure 1.3.7. Central pulse pressure waveform or augmentation index. Alx is the additional pressure added to the forward wave by the reflected wave. Alx is the ratio between augmentation pressure and central pulse pressure. The dicrotic notch represents closure of the aortic valve and is used to calculate ejection duration. Time to wave reflection is calculated at the point of rise in the initial ejection wave to the onset of the reflected wave. The reflected wave in this central pressure waveform results in augmentation of systolic flow (116).

Figure 1.3.8 shows that a pressure wave returning due to an increased pulse wave velocity will augment systole that can lead to increased cardiac loading and potentially lead to LVH (116). Endothelial dysfunction leads to increased peripheral vascular resistance and hence increased Alx. Patients with

diabetes also exhibit higher central augmentation pressures (118). This is another reason why LVH may be more prominent in diabetes.

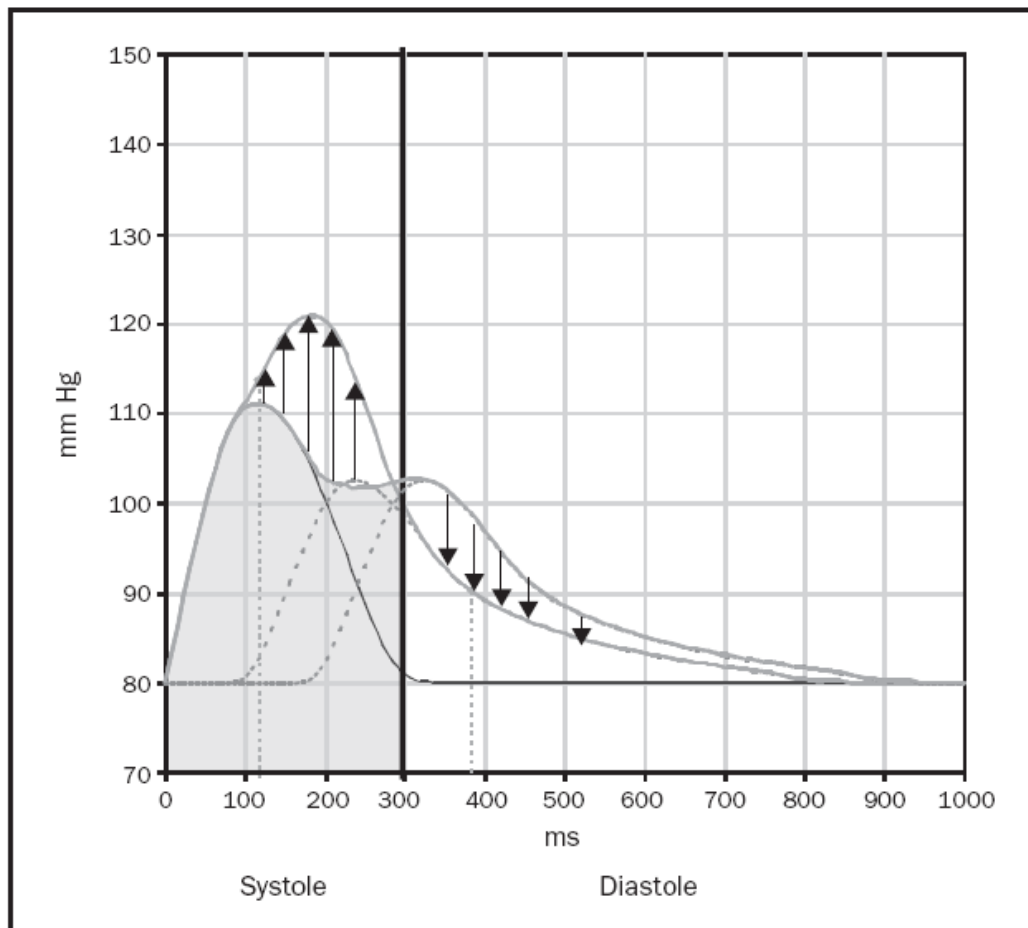


Figure 1.3.8. Effects of changes in LV workload on Alx. Reflection of the pulse wave during the systolic period leads to an increase in LV workload (black upward arrows) and a decrease in the diastolic pressure (black downward arrows). Reflection of the pulse wave during the diastolic period leads to a decrease in LV workload and an increase in diastolic pressure (116).

Various studies have correlated Alx with hypertension (119), diabetes (118), LVH (120), atherosclerosis (118) and diastolic dysfunction (121). Alx also

correlated better with worse CV outcomes than simple office BP in follow up studies (116). Therefore it has been suggested in the future it could be used as an additive to standard office BP to guide BP and other secondary prevention treatments, although it is not currently widely used in the United Kingdom.

iv. Pulse Wave Velocity

Pulse wave velocity (PWV) is part of applanation tonometry that relates to arterial vessel stiffness. It can be measured between the carotid arterial and radial or femoral artery. If the artery becomes stiffer in conditions such as atherosclerosis then the PWV will increase. Lehmann *et al* (1998) showed that PWV increases proportionally with increases in the number of risk factors patients have (122). PWV is also increased in advancing age and diabetes (123). Aortic stiffness has been correlated with worse CV outcomes in hypertensive patients (124).

v. Flow Mediated Dilatation and Prognostic Value of Endothelial Dysfunction

Measurement of endothelial function relies mainly on measuring 'endothelial dependent vasomotor tone' in response to pharmacological or physiological stresses. This tests the endothelial release of NO and other endothelial derived factors that cause dilatation of the artery. This can be then contrasted with 'endothelial independent' dilatation, such as caused by nitrates that are

usually given orally. Initial studies of endothelial function involved acetylcholine as the stimuli which was used because acetylcholine normally causes NO release (and other vasodilators) (112). Following on from this, studies tested endothelial function with other pharmacological endothelial agonists such as substance P and adenosine. Physiological responses to cold water testing and flow mediated dilatation (FMD) are other ways of unmasking physiological responses in the artery (125).

The main technique in the past was to measure venous occlusion plethysmography of the forearm, but this technique requires cannulation of the artery, making it invasive for the patient (126). Therefore an ultrasound technique of the brachial artery was developed in the 1990's to measure FMD in response to sheer arterial stress caused by occlusion of the artery (127). The original FMD studies showed that atherosclerotic arteries do not dilate in the same way as healthy arteries (125). FMD measures reactive hyperaemia caused by occlusion of the brachial artery that leads to local release of NO, and dilatation of the brachial artery. Again, this can be compared with sublingual administration of nitrates that cause 'endothelial independent' dilatation. There is a wide range of normal values ranging from 5% to 21%, and this may be due to individual methodology used (128). FMD has a number of advantages; it is cheap, non-invasive and well tolerated. It is also reproducible in the hands of trained operators (129). In contrast to this, it should be noted that Hardie *et al* (1997) found poor FMD reproducibility in healthy subjects (130), highlighting operators needing to be well trained and have experience in the technique which can be technically challenging at

times. Also, environmental factors influence the test, such as temperature, eating and caffeine, so adherence to a strict protocol of fasting before the test is important (128).

FMD is now established as a prognostic marker for CV disease, however not all studies have shown it to be independent of traditional risk factor assessment. Shimbo *et al* (2006) was the first to show in a prognostic study the association between FMD and CV outcomes in a patient group of multiple ethnicities (131). They included 842 patients without stroke or myocardial infarction and followed patients up at 36 months. Lower FMD levels predicted CV events (myocardial infarction, stroke and vascular death), HR 1.12 for every 1% decrease in FMD (95% CI, 1.01-1.25; p=0.03). The risk of events in patients with FMD in the lower two tertiles (FMD <7.5%) was significantly higher than those in the highest tertile, HR=3.28 (95% CI 1.07-10.06; p=0.04) for lowest versus highest tertile, and HR=3.05, (95% CI 1.03-9.66; p=0.04) for middle versus highest tertile. In their multivariate analysis including CV risk factors, the increase in risk associated with FMD was no longer statistically significant; therefore their findings are not independent of traditional CV risk factors. This was confirmed by Yeboah *et al* (2008) who found FMD in older patients added little to prognostic risk factor scoring, although it did predict future events (132).

Shechter *et al* (2007) and Rossi *et al* (2008) have been able to show a benefit over and above CV risk factors in predicting CV outcome with FMD. Shechter *et al* (2007) performed FMD prospectively on 110 patients, 46 had CV disease

and 64 where healthy controls (133). They found FMD was significantly lower in CV disease patients (9.5 +/- 8.0% vs. 13.5 +/- 8.0%, $P = 0.012$) compared to healthy controls (13.4 +/- 8.0% vs. 16.7 +/- 11.0%, $P = 0.084$; respectively). In addition, an inverse correlation between FMD and the number of traditional CV risk factors was found among all study patients. Mean follow-up was 15 months, and the composite CV endpoints (all-cause mortality, myocardial infarction, hospitalisation for heart failure or angina, stroke, coronary artery bypass grafting and percutaneous coronary interventions) were significantly more common in subjects with FMD less than 6% compared to subjects with FMD greater than 6% (33.3% vs. 12.1%, $P < 0.03$, respectively). Finally, Rossi *et al* (2008), studied a different cohort of patients and in much larger numbers (134). In total 2,264 post menopausal women were recruited for FMD with a mean follow up of 45 months. Risk adjusted RR values were 1.0, 1.33 (95% confidence interval [CI] 1.09 to 4.09), and 4.42 (95% CI 2.97 to 8.01) for women in the higher, intermediate, and lower tertile of FMD, respectively ($p < 0.0001$ for trend). The event rate for women in the lower tertile (FMD $\leq 4.5\%$) was greater than the combined event rate noted in the other two tertiles (women in the lower tertile accounted for 51 events [56.6% of total events]). When added to age and other conventional CV risk factors (smoking habits, presence of hypercholesterolemia, history of diabetes, hypertension), FMD contributed significantly to the model predicting CV events.

One pilot study has also shown that improving endothelial function may improve CV outcomes, but this was a small study on 68 patients with CAD

(135). In general terms, drugs used to treat CV risk factors i.e. lipid lowering strategies and ACE inhibitors do improve endothelial function (112). Allopurinol also improves endothelial function in various studies, and this is discussed later. In summary endothelial function does seem to predict future CV events, but it is not a mainstream test and its value over and above traditional risk factor evaluation is still under research. There are also limitations to the test as discussed.

d. Direct Affects of Glucose and Insulin on the Development of Left Ventricular Hypertrophy

Two studies have correlated increased plasma insulin levels with increased LVM. Verdecchia *et al* (1999) studied 101 non-diabetic patients with hypertension to determine the effects of insulin and insulin like growth factor (IGF-1) on LVM (136). IGF-1 and post load insulin accounted for over 40% of variability of LVM, after multivariate analysis, insulin and IGF-1 were powerful, independent predictors of LVM. A year later, Hirayama *et al* (2000) showed similar findings in 42 T2DM patients with raised LVM and plasma insulin levels (137). These patients also had normal office BP.

Rutter *et al* (2003) investigated the impact of insulin resistance on heart structure and function in a community based sample of Framingham study patients (65). Interestingly, hyperglycaemia correlated more closely with LVM in women than men and in their regression analysis they felt these changes could be accounted for by obesity. Importantly, they found that the more

severe glucose intolerance, the higher the LA size in both men and women. Lind *et al* (1996) also found a correlation between diabetes and LA size (66). Overall it appears that insulin resistance probably acts on the myocardium to cause LVH. This study confirms findings from other previous studies, which have all shown a correlation between LVM and insulin resistance (136, 138-141).

The potential mechanisms by which hyperinsulinaemia and insulin resistance are associated with LVH has been reviewed by Young *et al* (2002) (142). It has been known for some time that insulin exerts a growth stimulating effect on myocytes and also stimulated collagen synthesis in vascular smooth muscle (143, 144). Furthermore, insulin may lead to increased activity of the sympathetic nervous system, that may also proliferate myocardial cells (145). An overview of these potential mechanisms are elegantly summarised in Figure 1.3.9, by Rutter *et al* (2003) (65).

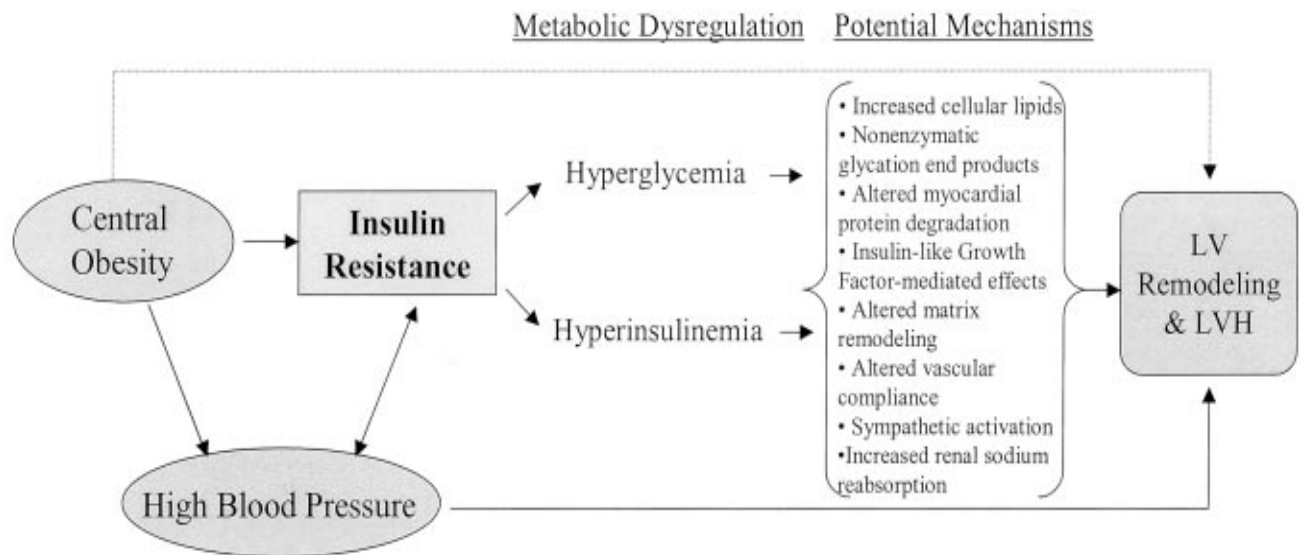


Figure 1.3.9. Potential mechanisms by which insulin resistance are associated with left ventricular hypertrophy(65).

Glucotoxicity has been implicated in the generation of ROS and insulin resistance and may thereby also affect cardiac gene expression (142). Fatty acids appear to do the same (142). Following on from this, glucose and fatty acids seem to be central to altered metabolic adaptation in response to pressure overload as well as the fact that glucose leads to transcriptional alterations of a number of genes (142). The end result of these transcriptional alterations is induction of foetal genes and substrate switching, enabling the heart to maintain cardiac output. It should be noted that in hearts without pressure overload there is also switching to foetal gene expression, possibly in a response to glucose (142). The concept of metabolic maladaptation, as discussed by Young *et al* (2002) encompasses three main concepts, as summarised in Figure 1.3.10. These are lipotoxicity, glucotoxicity and glucolipotoxicity (142). Lipotoxicity is the theory whereby a hyperlipid environment results in accumulation of lipids within the heart. This excess

accumulation of lipids can cause ROS, and apoptosis resulting in contractile dysfunction (142). Glucotoxicity is similar to lipotoxicity in that chronic hyperglycaemia leads to ROS formation. It also appears that hypertrophied and diabetic hearts there is insulin resistance as a result of chronic hyperglycaemia (142). Finally, glucolipotoxicity is the combination of these two processes, possibly accelerated by the suggestion that hyperglycaemia down regulates the expression of fatty acid metabolising genes, therefore accelerating the deposition of lipids and accelerating cardiac dysfunction (142).

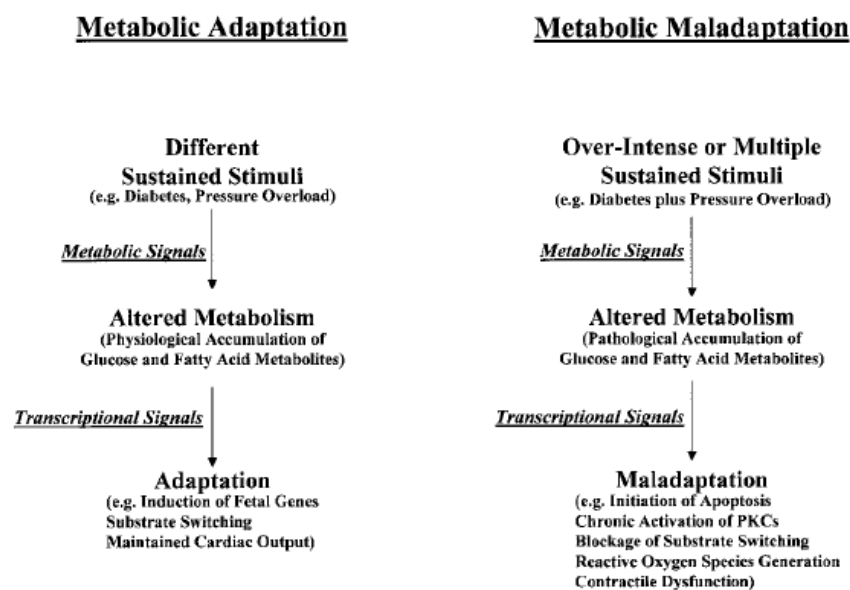


Figure 1.3.10. Summary of the possible mechanisms for metabolic adaptation and maladaptation of the heart (142).

e. Other Causes of Left Ventricular Hypertrophy

ii. Genetic Factors

Hypertension and LVH have a degree of genetic co-segregation (146). Various studies have found a link between a genetic predisposition and LVH, however cause and effect of LVH determinants with relation to genes can be difficult to establish, and are often described as 'complex traits'. It has been estimated that about 30% of LVH has a genetic component (147). Shigematsu Y *et al* 2005 found a genetic predisposition to LVH and insulin resistant that was independent of other traditional risk factors of LVH in a small cohort of 72 patients (148). Hong *et al* (2012) performed a genome-wide association study (GWAS) of ECG-LVH, in which the community-based Korea Association REsource (KARE) study (8432 controls and 398 cases) was analysed (149). There was consistent association with the 19q13.1 region that contains the RYR1 gene. Mutations in RYR1, which encodes a major calcium channel in the skeletal muscle, have been reported to correlate with CV diseases. Otherwise currently specific genes known to cause LVH directly are unknown.

ii. Renin Angiotensin System

The RAS seems to be important in the development of LVH, in particular angiotensin II. In rat models, angiotensin II causes myocyte hypertrophy (150). Angiotensin II is weakly correlated to LV mass, but when urinary

sodium excretion is also factored in, there is a strong correlation (151). Sodium excretion is an interesting addition to this study as it suggests that it is uncontrolled regulation of the RAS in humans may lead to changes in LVM, not only through hypertrophic effects of angiotensin II, but also through increased BP. It has also been shown that the angiotensin II receptor may be 'hyper-responsive' in some patients which also correlates with increased LVM (152). Uncontrolled RAS implies high RAS activation than would be expected for an individual's sodium status.

f. Brain Natriuretic Peptide in Left Ventricular Hypertrophy

Brain Natriuretic Peptide (BNP) is a neurohormone secreted by cardiac ventricles in response to stretch of cardiac myocytes. It was originally isolated from pigs' brains, hence the name (153). In general, any stress on the heart causing pressure or volume overload of the heart results in BNP release that is detectable in the blood. BNP acts in the kidney to cause excretion of sodium, blood vessels to cause smooth muscle relaxation and vasodilatation (154). BNP correlates with increasing LVM, and this has been shown in numerous patient groups including the elderly, hypertensive patients and the general population (155-158).

4. Treating Left Ventricular Hypertrophy

a. Benefits and Established ways of Regressing Left Ventricular Hypertrophy

Regressing LVH improves prognosis in patients with hypertension as detected by echo and the early studies did not focus on any particular agent to reduce BP and LVM. Verdecchia *et al* (1998) followed up 430 patients with essential hypertension, 26% of patients had echo LVH with an LVMI of 125g/m^2 or greater (159). Patients were treated with a combination of lifestyle and various anti-hypertensives to ensure BP was below 140/90. For patients who had a decrease in LVM from baseline there were fewer CV events compared with patients who had an increase in baseline LVM (1.78 vs. 3.03 per 100 person-years). In the small subset of patients with echo LVH, CV events were lower if there was a decrease in LVM (1.58 vs. 6.27 per 100 person-years). Importantly, this improved prognosis was independent of baseline LVM and BP and also the degree of BP reduction. Another small study by Koren *et al* (2002) found similar findings in 172 patients with essential hypertension (160). In this trial anti-hypertensives were prescribed at the discretion of the physician. Fewer of the 91 patients with unchanged or decreased LVM experienced CV events than the 81 patients whose LVM increased during follow-up (8.8% vs. 19.8%). Absence or presence of LVH on the follow-up echo was the strongest predictor subsequent CV events. Verdecchia *et al* (2003) confirmed these initial findings with a meta-analysis of four trials (161). They found that regressing LVH with antihypertensives is associated with a

significant reduction in CV events (OR 0.41, 95% CI 0.21 – 0.78). More recently Pierdomenico *et al* (2008) confirmed these early studies that regressing LVH over a two year period with any anti-hypertensive to lower BP reduces CV risk (RR 0.36, 95% CI 0.19 – 0.68) (162). This was the case regardless of ECG findings of LVH.

Klingbeil *et al* (2003) performed a meta analysis of 80 double blind randomised clinical trials to study the effect of different anti-hypertensives on LVM (79). In total, 3,767 patients were included in the active arm and 346 in the placebo arm. The results showed that after adjustment for treatment duration and change in diastolic BP, there was a significant difference ($P = 0.004$) among medication classes. LVMI decreased by 13% with ARB's (95% CI, 8% - 18%), by 11% with calcium antagonists (95% CI, 9% - 13%), by 10% with ACE inhibitors (95% CI, 8% - 12%), by 8% with diuretics (95% CI, 5% - 10%), and by 6% with beta-blockers (95% CI, 3% - 8%). In pair wise comparisons, ARB's, calcium antagonists, and ACE inhibitors were more effective at reducing LVM than were beta-blockers.

The Losartan Intervention For Endpoint reduction in hypertension (LIFE) study and the Heart Outcomes Prevention Evaluation (HOPE) study have specifically looked at reducing BP with ARBs and ACE inhibitors in much larger numbers of patients, in contrast to the studies discussed above, that both included a variety of anti-hypertensives to reduce blood BP. Many subgroup analyses have been published in relation to these two trials therefore they will be dealt with separately.

i. Losartan Intervention For Endpoint Reduction in Hypertension (LIFE) Study

The LIFE trial was designed in the 1990's to answer the question as to which anti-hypertensive, in this case the ARB losartan, is most effective at reducing morbidity and mortality in hypertensive patients and signs of ECG LVH (163). Losartan based therapy was compared with atenolol based therapy in patients with hypertension and evidence of LVH from ECG criteria. The LIFE trial was a double masked, randomised parallel group study of 9,193 patients aged 55-80. 1,195 patients (13%) of patients recruited had diabetes and 86% were white. Mean follow up was 4.8 years. The fall in BP was similar in both the losartan and atenolol arms, but despite this the primary composite endpoint occurred for losartan was 23.8 per 1,000 patient-years and atenolol was 27.9 per 1,000 patient-years (RR 0.87, 95% CI 0.77 - 0.98). Losartan was better than atenolol in terms of CV mortality (RR was 0.89, 95% CI 0.73-1.07) with 232 and 309, fatal or non-fatal strokes (RR 0.75, 95% CI 0.63-0.89) and myocardial infarctions (RR 1.07, 95% CI 0.88-1.31). There were also improved ECG criteria for LVH in the losartan arm. Because of the similar falls in BP with both atenolol and losartan this raises the question of what losartan does differently to atenolol. It has been suggested that losartan may regress LVH more aggressively, because angiotensin II may have detrimental effects itself or because losartan has specific effects that are still unknown. Interestingly and never fully accounted for, the incidence for new-onset diabetes was less frequent with losartan by 25% (163). Okin *et al* (2003) specifically analysed the ECG's of all the patients in LIFE and found that

losartan resulted in greater LVH regression than atenolol (164). Importantly, this finding holds true for echo LVH as well (165).

In a sub group analysis of LIFE, Devereaux *et al* (2002) performed serial echos on 754 patients with ECG LVH to assess LVH regression (77). They found that LVM decreased after sustained BP reduction of at least two years. There was a small, and not significant, fall in BP after one year. This suggests that at least two years treatment is needed to see the full benefits of anti-hypertensive treatment and also that the benefits are seen after sustained BP control.

The onset of diabetes in was studied in more depth at a later date by Okin *et al* (2007) (166). A sub-set of 7,998 LIFE patients without diabetes were followed up with serial ECGs to see who developed diabetes. Resolution or absence of ECG LVH criteria was associated with a lower incidence of diabetes, even after adjusting for losartan and other risk factors for diabetes. Analysis of 1,195 patients in the LIFE trial with diabetes confirmed the original findings of the trial that losartan is more effective than atenolol at reducing CV morbidity and mortality in patients with diabetes, ECG LVH and hypertension (167). Further analysis of LIFE has shown that in patients with diabetes and established ECG LVH that reduction in BP does not regress LVH to the same extent as in a non-diabetic (168). This may explain in part why despite BP treatment, patients with diabetes still fair worse in terms of CV morbidity and mortality.

Over the last decade, sub group analyses of LIFE have shown that losartan has beneficial effects over atenolol in a number of outcomes. Losartan has beneficial effects on CV risk, hospitalisation for heart failure, sudden cardiac death, new onset AF, stroke and major CV events (162, 169-172).

Finally an interesting sub-study from 2004 looked at the effects of uric acid in the LIFE study (173). The increase in uric over 4.8 years in the LIFE study was attenuated by losartan compared with atenolol treatment, appearing to explain 29% of the treatment effect on the primary composite end point. The association between uric acid and events was stronger in women than in men (173).

ii. The Heart Outcomes Prevention Evaluation (HOPE) Study

The HOPE study does look specifically at LVH, but included high risk patients (n=9,297) without heart failure (174). The cohort included 9,297 patients in total and of these 8,162 had CV disease, 4,355 had hypertension and 3,777 had diabetes. Ramipril (10mg) was compared with placebo and the primary outcome was the composite endpoint of myocardial infarction, stroke or death from CV causes. The primary endpoint was reached in 14% of the ramipril group and 17.8% in the placebo group, relative risk reduction 0.78 (95% CI 0.70 – 0.86, $p < 0.001$). Significantly fewer patients in the ramipril group had a cardiac arrest, worsening angina, heart failure or a new diagnosis of diabetes. These differences were seen after correcting for diabetes, age, and most importantly hypertension.

The Effects of Ramipril on Cardiovascular and Micro-vascular Outcomes in People with Diabetes Mellitus (MICRO HOPE) study should be mentioned here because it specifically looked at the sub-group of patients with diabetes recruited in HOPE (n=3,577) (78). Ramipril reduced the risk of the combined primary outcome of myocardial infarction, stroke or CV death in diabetic patients by 25%. After adjustment for BP changes, the results remained significant.

In summary although ECG is not the most reliable way to look for LVH, if a patient is hypertensive and have ECG and/or echo LVH then the blockade of the RAS should be considered along with tight BP control. The benefits extend to people with diabetes and blockade of the RAS also has beneficial effects on the development of new onset diabetes.

iii. Regressing Left Ventricular Hypertrophy in the Context of Normal Blood Pressure

The data on regressing LVH in the context of normal BP is limited. The HOPE trial is of particular relevance because of later sub-group analyses of patients (n= 8,281) who had two comparable ECG's over the study period and a small group who had echo (175, 176). Because some patients included did not have clinical hypertension this gives us unique information about the effects of ramipril in patients with ECG LVH and normal BP. In total, 676 patients had baseline LVH on ECG. In the group of patients who had regression or prevention of LVH, in the ramipril arm 12.3% reached the primary end point of

CV death, myocardial infarction or stroke, compared with 15.8% in the placebo arm. There was also a reduction in heart failure. These changes were independent of BP. Lonn *et al* (2004) echoed 506 patients with normal BP (baseline average BP 131/76) to quantify LVM (176). After four years of treatment, LVM increased in the placebo group by $3.98 \pm 2.08 \text{g/m}^2$ and by $4.16 \pm 1.86 \text{g/m}^2$ in the ramipril 2.5mg group. In the group of patients who received 10mg ramipril it decreased by $2.02 \pm 2.25 \text{g/m}^2$. This sub-study is interesting because it suggests that ramipril reduces LVM by other means than just BP, possibly due to angiotensin II effects, which were discussed in detail previously.

A small study by Nielsen *et al* (1998) studied the effect of ramipril compared to placebo in 38 patients with normal BP and T2DM with no microalbuminuria (177). BP was almost identical at baseline ($132/76 \pm 3/1$ vs. $133/74 \pm 5/2 \text{mmHg}$) and remained stable during follow-up ($134/76 \pm 3/1$ vs. $136/74 \pm 6/2 \text{mmHg}$) in the ramipril and placebo groups respectively. LVM was comparable at baseline and decreased significantly more in the ramipril group (17.6 ± 3.0) as compared with the placebo group ($5.7 \pm 4.6 \text{g/m}^2$).

iv. Non-Pharmacological Ways of Regressing Left Ventricular Hypertrophy

Weight loss and dietary sodium restriction have been shown to be effective in regressing LVH in a small number of studies. MacMahon *et al* (1986) studied 41 men with hypertension and obesity and found that weight loss on average

of 8.3kg over a 21-week period resulted in reduced LV dimension when compared to placebo. This was independent of BP.

The Treatment of Mild Hypertension Study (TOMHS) included 844 patients in a double-blind, placebo-controlled clinical trial mildly hypertensive patients randomised to nutritional-hygienic intervention plus placebo or nutritional-hygienic intervention plus one of five classes of antihypertensive agents including a diuretic (chlorthalidone), beta-blocker (acebutolol), alpha-antagonist (doxazosin), calcium antagonist (amlodipine), or an ACE inhibitor (enalapril) (178). Serial echos were performed over four years. Changes in blood pressure averaged 16/12mmHg in the active treatment groups and 9/9 mmHg in the nutritional-hygienic intervention only group. All groups showed significant decreases in LVM from baseline that appeared at three months and continued for 48 months. Changes in weight, urinary sodium excretion, and systolic BP were moderately correlated with changes in LVM.

Jula *et al* (1994) also studied the effects on LVH of a non-pharmacological treatment program based mainly on sodium restriction and weight loss (179). The study was small (n=76) and the patients were un-treated hypertensives who were followed up with serial echo for one year. The daily sodium excretion, BP and weight decreased significantly in the treatment group. After one year of sodium restriction, LVM decreased by 5.4% and LVMI decreased by 4.7% whereas no changes occurred in these parameters in the control group.

b. Potential Ways of Regressing Left Ventricular Hypertrophy

The strategies described above are only partly effective. Mancia *et al* (2002) studied 2,051 people involved in the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) study population (180). They found that LVH persists in about 20% of hypertensives who attain target BP, therefore LVH in patients with controlled BP is common and it would seem blockade of the RAS and anti-hypertensives do not hold all the answers in terms of reducing the risk from LVH. Furthermore, LVH carries the same risk in normotensive individuals as in those with high BP. Brown *et al* (2000) showed that during 16.8 years follow up of hypertensive patients, after adjustment, normotensives with LVH had survival similar to hypertensive adults with LVH. They also had lower survival than normotensive and hypertensive adults with no LVH (181). Since LVH regression is effective regardless of BP we thought it valuable to study the XO inhibitor allopurinol as a potential way of decreasing LVM in diabetic patients with LVH and normal BP.

5. **Xanthine Oxidase and Allopurinol**

a. **History of the Xanthine Oxidase Inhibitor Allopurinol**

Allopurinol is a XO inhibitor approved for use in Britain by the Food and Drug Administration in 1966. Allopurinol is the mainstay of treatment for gout and is also indicated for uric acid and calcium oxalate stones and prophylaxis of hyperuricaemia associated with chemotherapy agents (182).

Allopurinol was originally synthesised as an attempt to increase the efficacy of new antineoplastic agents in the mid-1950s, such as 6-mercaptopurine (6-MP) (183). 6-MP was being investigated at the Sloan-Kettering Institute by the eventual Nobel Prize winners Gertrude Elion and George Hitchings for its anti-cancer properties. 6-MP was showing promise as a drug that could be used in the fight against leukaemia as it was displaying very high activity against leukaemia in children (184). The enzyme XO was being studied as part of these experiments, as inhibiting XO was thought to potentiate the anti tumour properties of 6-MP. Elion and Hitchings were experimenting with a number of drugs to inhibit XO to potentiate 6-MP and one of these was allopurinol. It was during these experiments that the potential XO inhibitor properties of allopurinol were discovered, as it was already known that XO was involved in the formation of uric acid from xanthine. Subsequent tests showed effective reduction of urinary and serum uric acid with allopurinol (183, 184). Figure 1.5.1 shows that XO mediates the conversion of hypoxanthine to xanthine and xanthine to uric acid (183).

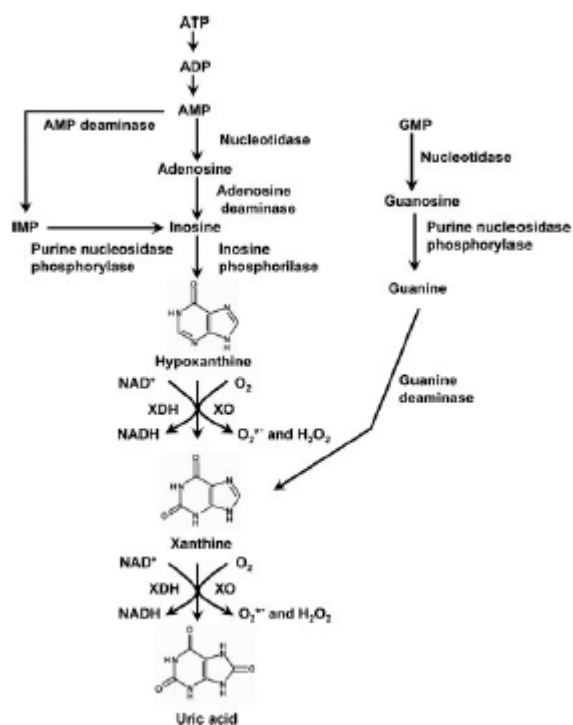


Figure 1.5.1 Purine degradation pathway (184).

In 1988 the discovery of 6-MP and allopurinol led Gertrude Elion and George Hitchings to be awarded the Nobel Prize in Physiology and Medicine (184, 185). Interestingly this Nobel Prize was shared with the former Chancellor of Dundee University Sir James Black who developed the first clinically useful beta blocker, propranolol, in 1964. The discovery of allopurinol was presented in 1988 Nobel Lecture the 'Purine Path to Chemotherapy' (185). Since its discovery, allopurinol has been the mainstay of the treatment of gout for nearly fifty years. In recent times, exciting new discoveries have found new uses for allopurinol in patients with CV disease, which is discussed later in this thesis (186).

b. Xanthine Oxidase

i. Structure

Before we consider the biochemistry of allopurinol it is important to understand the role of XO and xanthine dehydrogenase (XDH) in the Purine degradation pathway, Figure 1.5.1. XO and XDH are interconvertible forms of the same enzyme known as Xanthine Oxidoreductase (XOR) (187). XOR was first identified 100 years ago in milk and belongs to the family of enzymes called molybdoenzymes which also includes aldehyde oxidase and sulphite oxidase (188). In man the enzyme is found in the dehydrogenase form and has been most extensively studied in bovine milk. Both enzymes are structurally similar; they have identical x-ray absorption spectra because they have the same ligand environment and co-ordinating geometry around a molybdenum centre (184). These two forms of XOR can be inter-converted irreversibly by sulphide reagents or reversibly from XDH to XO by proteolysis (187). Harrison *et al* (2002) describes the XOR structure as consisting of a homodimer of approximately 300kDa. Each subunit is identical and contains four redox centres, a molybdenum co-factor (Mo-co), one FAD and two Fe₂S₂ sites, see Figure 1.5.2. The Mo-co comprises an organic pterin derivative called molybdopterin, containing a cyclised dithiolene side chain with one Mo atom (187). Each of the subunits acts independently as a catalyst.

The crystal structure of the XDH dimer divided into the three major domains and two connecting loops is shown in Figure 1.5.3. The hydroxylation of xanthine takes place at the molybdopterin centre and the other electrons introduced are rapidly transferred to the other linearly aligned redox centres (184).

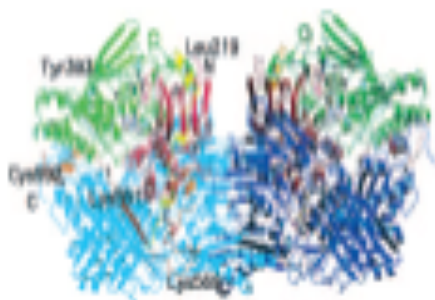


Figure 1.5.3. Crystal structure of xanthine dehydrogenase (184).

ii. Actions

Both XO and XDH catalyse the final stages of purine metabolism, namely hypoxanthine to xanthine and xanthine to uric acid (188). During this process XOR produces the ROS O_2^- and H_2O_2 , by reduction of molecular oxygen when hypoxanthine is catalysed to xanthine. XOR also catalyses the hydroxygenation of N-heterocyclic and aldehyde substrates through the Mo site. At the Mo site NADH donates its electrons to FAD which then in turn forms either NADH from NAD^+ or the ROS O_2^- and H_2O_2 from oxygen (187). XO only reduces oxygen whereas XDH can reduce both oxygen and NAD^+ (188). This is shown schematically in figure 1.5.4.

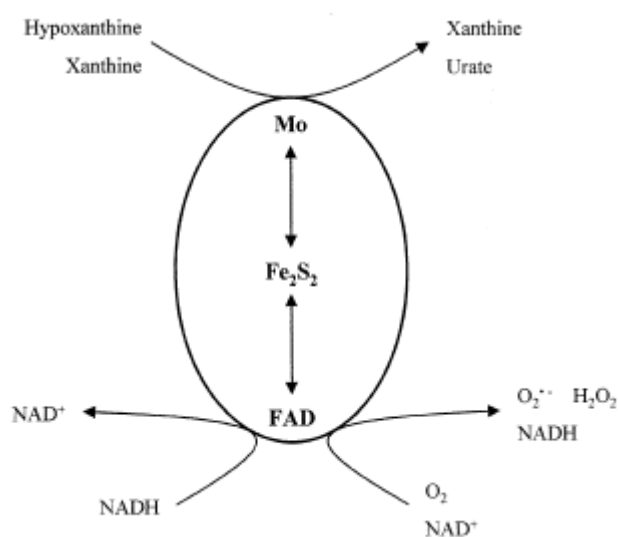


Figure 1.5.4. Formation of reactive oxygen species and NADH from the XOR catalysed oxidation of xanthine and hypoxanthine (187).

The mechanism of how XOR reacts with xanthine to oxidise oxygen is complex and some aspects are uncertain (184, 187-189). Xia *et al* (1999)

investigated the mechanism of how XOR reacts with xanthine. They found that the reductive half reduction occurs at Mo-co where XOR accepts two electrons from xanthine, reducing Mo (VI) to Mo (IV), as shown in Figure 1.5.5 (189). Hille R *et al* (1981) showed that a fully reduced XOR transfers electrons to generate H_2O_2 . XOR then transfers its remaining electrons in separate steps with each electron independently reducing oxygen to produce O_2^- , as shown in Figure 1.5.5 (190).

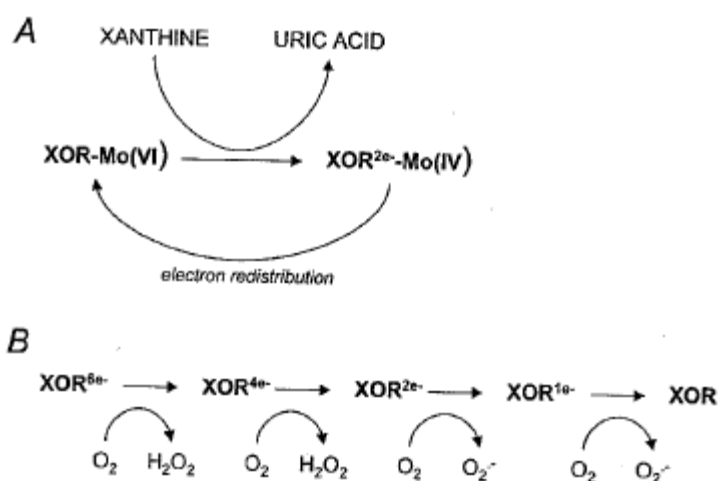


Figure 1.5.5. Mechanism of XOR reaction with xanthine (188).

XOR has also been shown to catalyse the reduction of nitrates to nitrites and nitrites to NO (187). Under anaerobic conditions and in the presence of nitrite and xanthine or NADH, NO is generated at the Mo site. In the presence of molecular oxygen this is reduced at the FAD to give O_2^- which reacts rapidly with NO to give peroxynitrite (187), shown in Figure 1.5.6. The formation of ROS, NO and peroxynitrite are important in endothelial function and the pathophysiology of XOR and are discussed below.

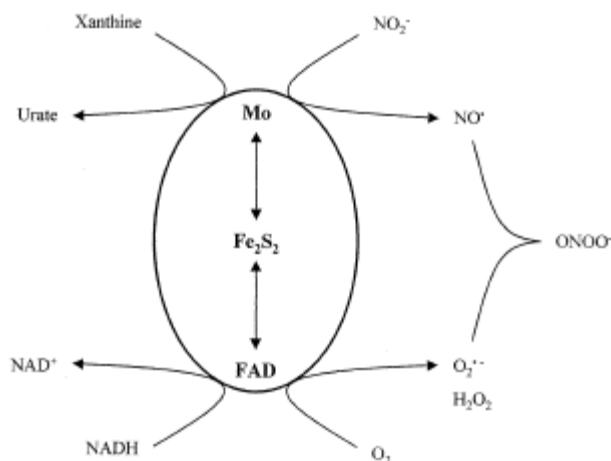


Figure 1.5.6 XOR catalysed production of peroxynitrite (187).

iii. Distribution

XOR has been found in all species studied to date, but there is variation in the distribution amongst these species. XOR is studied using immunocytochemistry which can recognise inactive and active forms, therefore studies looking at distribution have been inconsistent (191). In man XOR activity is found in the highest levels in the liver and intestine, with activity present in most other tissues studied including the lung, kidney, heart, brain and plasma (191, 192). The presence of XOR in cardiac tissue has been studied by Muxfeldt *et al* (1987) (193) and Bruder *et al* (1983) (194). Plasma XOR, or circulating XOR, has been reported in varying quantities around human bodies and has also been found to be higher in certain disease states including liver damage (195, 196). Most notably higher levels of circulating XOR have been found in rheumatoid arthritis, mixed connective tissue disease, scleroderma and atherosclerosis (187, 197). XOR has also been shown to circulate around the body in higher levels in liver ischaemia

and reperfusion, haemorrhagic shock, during thoracic surgery, liver transplantation and hind limb ischaemia and reperfusion (184). There is evidence that circulating XOR triggers endothelial dysfunction at remote sites, such as lung tissue, mediating tissue damage in these conditions. Circulating XOR binds to endothelial cells via glycosaminoglycans where it acquires higher oxidant producing capacity and increased stability (198). It may be that this circulating XOR is more important in the pathophysiology of endothelial dysfunction than XOR produced from endothelial cells (199, 200).

The gene encoding human XOR is located on the short arm of chromosome 2 and comprises 36 exons (188). A number of physiological conditions have been shown to affect transcription of the XOR gene and are summarised in Table 1.5.1.

| Positive regulators | Negative regulators |
|---------------------------------|----------------------------|
| Hypoxia | Hyperoxia |
| Lipopolysaccharide | |
| Interferon γ | |
| Interleukin – 1 | |
| Interleukin – 6 | |
| Tumour necrosis factor α | |
| Dexamethasone | |
| Cortisol | |
| Prolactin | |

Table 1.5.1. Regulation of XOR gene expression (188).

iv. Possible Pathophysiological Roles of XOR

ROS generated by XOR have been studied as causal mechanisms of tissue damage. ROS cause injury via the production of superoxide and hydrogen peroxide that undergo further reactions to form highly active hydroxyl free radicals (Haber-Weiss Reaction) and peroxynitrite, described above. These free radicals cause lipid peroxidation and DNA and amino acid oxidation. This results in disruption of the membrane architecture and lysosomal enzyme release, genetic mutations and enzyme dysfunction (188). These pathological process have led XOR to be implicated in myocarditis, hypertension and heart failure at the level of the endothelium (188).

The ROS produced by XOR may be important in the pathophysiology of heart failure through the process of mechanoenergetic uncoupling. Mechanoenergetic uncoupling in heart failure describes worsening LV function in the setting of unchanged oxygen demand by the myocardium i.e. the heart requires extra oxygen to maintain the same mechanical function. This leads to a fall in the mechanical contractibility of the myocardium (201). At the level of the myofilament ROS may decrease the responsiveness to calcium activation and may also increase oxygen consumption by the whole heart. It is postulated these two mechanisms worsen mechanoenergetic uncoupling in heart failure (201).

The concept of ischaemia reperfusion injury (IR) was first introduced by Granger *et al* (1981) (202). Granger *et al* (1981) investigated the idea that

XOR generated ROS cause ischaemic bowel injury due to ATP catalysation during ischaemia. When the tissue is reperfused there is generation of O_2^- and H_2O_2 when the readmitted oxygen, hypoxanthine and XO combine (202), shown in Figure 1.5.7.

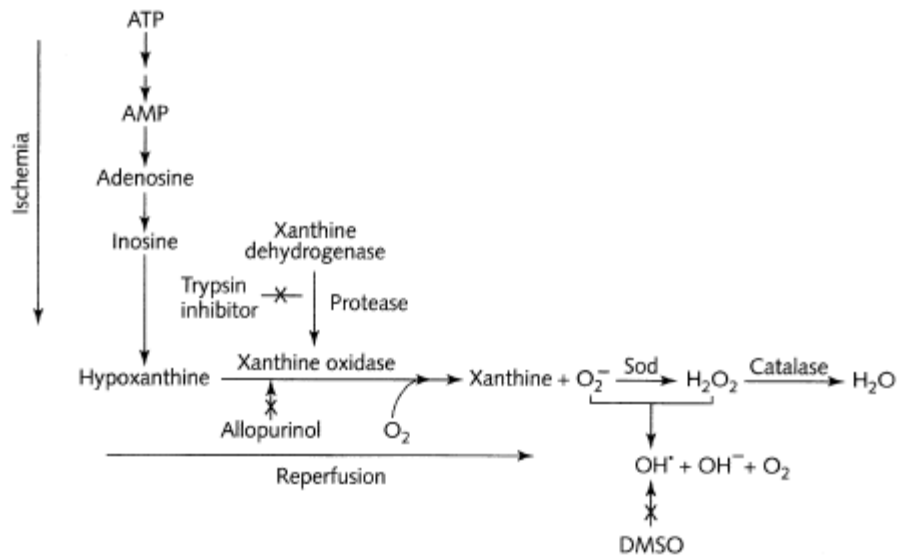


Figure 1.5.7. Mechanism of ROS during ischaemia reperfusion injury (187).

These oxygen derived ROS may also play an important role in the pathology of myocardial dysfunction after ischaemic events (203). The idea that IR plays a role in tissue damage led to research into the role of allopurinol in attenuating endothelial damage in a number of tissues. As mentioned before there are low levels of XOR activity in rabbit, pig and human hearts so the role of XOR mediated injury in IR is open to debate (191, 192). It is possible the circulating XOR that binds to endothelial tissue is released by XOR rich tissues such as the liver that provides the XOR in IR. The concept of circulating XOR is discussed above. The idea of XOR interacting with neutrophils that are known to invade post ischaemic tissue has also been

researched at a micro vascular level (204). Granger *et al* (1981) studied whether XOR derived oxidants play a role in leukocyte microvascular interactions initiated by IR in cat intestine. Their findings showed there was some evidence XOR derived ROS initiate leukocyte reperfusion in IR intestine (204).

These IR interactions and the involvement of neutrophils are important in understanding the possible therapeutic benefits of allopurinol not just in IR but in other vascular pathologies.

c. Biochemistry of Allopurinol

Allopurinol is a weak acid that is rapidly converted to oxypurinol by the enzyme aldehyde oxoreductase. The chemical structure of allopurinol is 1,5-dihydro-4H-pyrazole [3,4-*d*] pyrimidin-4-one, as shown in Figure 1.5.8. Allopurinol is an analogue of hypoxanthine and oxypurinol is an analogue of xanthine. Oxypurinol is a non-competitive inhibitor of XO. Oxypurinol has a longer half life and persists for longer in tissues than allopurinol, hence it is the formation of oxypurinol that is responsible for the most of the pharmacological activity of allopurinol (205).

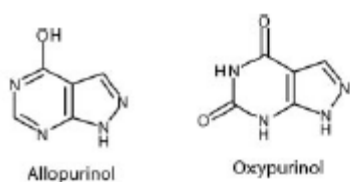


Figure 1.5.8. Chemical structures of allopurinol and oxypurinol (184).

d. Clinical Pharmacokinetics of Allopurinol

In animals allopurinol is found in highest concentrations in vascular tissue, blood, liver, intestine and the heart. Allopurinol binds negligibly to plasma proteins (206). Allopurinol is well absorbed from the gastrointestinal tract after an oral dose but it is poorly absorbed from the rectum (207),(206). Breithaupt *et al* (1982) used a high pressure liquid chromatographic method to measure allopurinol and oxypurinol absorption orally and intravenously. They measured allopurinol in the plasma and urine of six healthy volunteers after single doses of oral and intravenous allopurinol (208). The total recovery from urine was 77% (allopurinol 8%, oxypurinol 69%) after 300mg oral allopurinol. 88% was recovered from the urine when 300mg allopurinol was given intravenously. They found only 10% of allopurinol is not absorbed when given via the oral route compared to intravenously (208).

The mean half life of allopurinol has been measured at between one to two hours, compared to the half life of oxypurinol of 16 to 23 hours in various different studies (205) (207). The maximum concentration of allopurinol has been shown to be achieved after about one hour and 60% to 70% of allopurinol is rapidly converted to oxypurinol by XO (183, 206). In summary the pharmacological activity of allopurinol is due largely to oxypurinol, as a large proportion of allopurinol is converted to oxypurinol that has a much longer half life (205).

The pharmacokinetic parameters of allopurinol, with normal renal function, after oral dosage from Day *et al* (2007) are as follows (205):

Bioavailability of 79 +/- 20%

Elimination half life 1.2 +/-0.3 hours

Apparent oral clearance of 15.8 +/- 5.2mL/min/kg

Apparent volume of distribution after oral administration of 1.31 +/- 0.41
L/kg

Day *et al* (2007) showed that 90mg of oxypurinol is formed from every 100mg of allopurinol. Based on their calculations the pharmacokinetic parameters of oxypurinol, with normal renal function, after oral dosage from Day *et al* (2007) are as follows (205):

Elimination half life 23.3 +/- 6 hours

Apparent oral clearance of 0.31 +/- 0.07mL/min/kg

Apparent volume of distribution after oral administration of 0.59 +/- 0.16
L/kg

Oxypurinol is primarily excreted by the kidneys, whereas allopurinol is eliminated by metabolism (209). After glomerular filtration, oxypurinol is absorbed in the renal tubules, which is the main reason why oxypurinol has a longer half life than allopurinol (210). Renal tubular absorption of oxypurinol is affected by changes in urine pH, glomerular filtration and uric acid concentrations (183). It follows that allopurinol elimination is not reduced with age because it is eliminated by metabolism, whereas oxypurinol elimination is

reduced in the elderly because of age dependent decline on renal function (209).

e. Dose response studies of allopurinol

Graham *et al* (1996) studied the dose response of allopurinol and how it relates to the steady state oxypurinol concentration. They found that the oxypurinol concentration in steady state increased over the dosage range of allopurinol from 50mg to 600mg in a linear fashion. This linear increase in oxypurinol concentration did not occur between 600mg and 900mg of allopurinol. This is possibly due to saturation of XO or the saturation of the renal tubular absorption of oxypurinol (183). The dose response studies performed by Graham *et al* (1996) are summarised in Table 1.5.2.

| Allopurinol dose per day (mg) | Steady state oxypurinol concentration (mg/L) |
|----------------------------------|---|
| 50 | 1.77 +/- 1.59 |
| 100 | 2.67 +/- 1.59 |
| 300 | 5.59 +/- 1.5 |
| 600 | 9.56 +/- 1.92 |
| 900 | 12.21 +/- 2.13 |

Table 1.5.2. Oxypurinol dose response table (183).

f. Clinical Pharmacodynamics of Allopurinol

Allopurinol is generally safe with known side effects, and as mentioned previously, has been used for over 40 years. Caution should be used in hepatic and renal impairment. Pregnancy and breast feeding is a contra-indication for use. Rashes and gastrointestinal disorders are the commonest side effects. Hypersensitivity reactions occur rarely and include exfoliation, fever, lymphadenopathy, arthralgia, and eosinophilia resembling Stevens-Johnson or Lyell's syndrome. The BNF lists malaise, headache, vertigo, drowsiness, visual and taste disturbances, hypertension, alopecia, hepatotoxicity, paraesthesia and neuropathy, gynaecomastia, blood disorders (including leucopenia, thrombocytopenia, haemolytic anaemia and aplastic anaemia) as being very rare (182).

Allopurinol enhances the effects and increases the toxicity of azathioprine and mercaptopurine. Allopurinol may enhance the anticoagulant effects of warfarin (182). Allopurinol increases the plasma concentration of didanosine, ciclosporin and theophylline (182).

g. Allopurinol as an 'Antioxidant'

Various studies dating back to the 1990s have shown that high uric acid levels are associated with a worse prognosis in patients with CV disease (211). Allopurinol has been shown in various clinical trials to be potentially beneficial in reducing OS and improving endothelial function in a number of conditions in

patients with CV disease, including diabetes (212), CAD (213), chronic heart failure (214) and smokers (215). However, none of these trials have shown reduced CV events as an endpoint because they were small trials. As discussed above XO catalyses the formation of urate and also catalyses the formation of ROS. It is thought these ROS species may increase CV risk, mechanoenergetic uncoupling and IR injury as discussed before. It is important to realise that allopurinol prevents the formation of OS which is very different from anti-oxidant vitamins which attempt to mop up already formed OS.

i. Allopurinol may Improve Mechanoenergetic Uncoupling

The postulated role XOR plays in mechanoenergetic uncoupling is discussed above and Ekelund *et al* (1999) therefore sought to test whether allopurinol improves mechanoenergetic uncoupling in dogs. The dogs had induced heart failure by means of a pacemaker and allopurinol was infused intravenously. They showed that allopurinol improved the myocontractility and decreased myocardial oxygen demand possibly through its effects as an XOR inhibitor thereby reducing the generation of ROS (216).

The potential use of allopurinol in dilated cardiomyopathy has also been studied on a small scale by Cappola *et al* (2001). They based their study on the theory that ROS generated by XOR may contribute to imbalance between myocardial energy consumption (energetics) and LV performance (mechanical function) i.e. mechanoenergetic uncoupling as described before.

The allopurinol was given as an infusion into the left coronary artery and haemodynamic pressure measurements to quantify LV function were made by cardiac catheterisation to measure myocardial oxygen consumption, peak rate of rise of LV pressure, stroke work and efficiency. The study was small only involving nine patients. Inhibition of XOR by allopurinol improved myocardial oxygen demand $22\pm 9\%$ and efficiency $40\pm 17\%$. These effects seem to be apparent even with ACE inhibitors and beta-blockers, therefore allopurinol may have potential benefits in the future in the treatment of heart failure (217).

ii. Allopurinol as a Free Radical Scavenger in Animal Models

Early studies in the 1980s looked at free radicals in animals. In 1987 it was reported that allopurinol and oxypurinol scavenged free radicals in a pig heart, which do not contain XO (neither does pig blood) (218). Therefore it would seem allopurinol and oxypurinol exert other non XOR mechanisms such as they may scavenge free radicals in the IR myocardium by themselves, independent of inhibiting XO (218). Further research by Hoey *et al* (1988) also added to the idea of allopurinol and oxypurinol scavenging free radicals directly in animals tissues not containing XO (219). Again, this was a study of IR in animal models.

Malkiel *et al* (1993) studied the interaction of allopurinol with copper. Copper can participate in single electron reactions and mediate the formation of ROS (220). They found allopurinol formed a complex with copper that reduced the copper mediated and ascorbate driven DNA breakage. Essentially it seems

allopurinol acted as a chelator with the copper ions and prevented DNA damage which is another possible mechanism whereby allopurinol acts as an anti-oxidant (220).

iii. Evidence Allopurinol may be Beneficial in Ischaemia Reperfusion Injury

Various studies have looked at outcomes in humans given allopurinol e.g. in post myocardial infarction and in post coronary artery bypass grafting patients. One of the theories behind the injury in these patients is the IR model discussed before. Sisto *et al* (1995) randomised 81 patients to 600mg allopurinol and vitamin E and C treatment or placebo before they underwent coronary artery bypass grafting to see if there was an improvement in myocardial events after reperfusion in coronary artery bypass grafting. The allopurinol treated patients needed less dopamine support, had fewer peri-operative infarctions and had fewer ischaemic events on ECG monitoring (203). Further studies have shown positive effects when using allopurinol to improve IR after coronary artery bypass surgery. Johnson *et al* (1991) found that hospital mortality fell from 18% to 4% in their small, randomised cohort of 169 patients in the early 1990s. Again the need for inotropic support was reduced in this study (221). Johnson *et al* (1991) has gone as far as to say in their institute in Milwaukee that allopurinol is administered to all patients undergoing coronary artery bypass grafting unless contraindicated. These studies are small and therefore inconclusive and allopurinol is not a routine treatment for these patients in other centres.

Promising findings have been shown looking at CK-MB activity, granulocyte counts and malondialdehyde activity during the ischaemic period of coronary artery bypass grafting (222, 223). The idea that lipid peroxidation may be reduced in coronary artery bypass grafting patients has been studied on a small scale by Belboul *et al* (2001) and Movahed *et al* (1996). The theory is that lipid peroxidation is triggered by granulocyte activation via the complement system as a result of the by products of IR. Belboul *et al* (2001) found allopurinol reduced malondialdehyde activity (a marker of ROS) and lipid peroxidation in small numbers of patients undergoing open heart surgery (223). Movahed *et al* (1996) also found an improvement in lipid peroxidation products and CK-MB activity with allopurinol (222). These small studies add to the idea that ROS species generated by IR may be reduced by allopurinol and have potentially beneficial effects, but neither of these two studies correlated reduced ROS activity with improved clinical outcomes.

Another postulated mechanism for tissue damage in IR is the activation of heat shock factor 1 as it may be that heat shock factor plays a myocardial protective role in IR (224). Nishizawa J *et al* (1999) used rat hearts to induce IR. They found that ROS species generated played a role in the gene transcription of heat shock factor 1 and this transcription was reduced with allopurinol. This provides another theoretical way that allopurinol may protect the heart against IR injury (224).

iv Allopurinol may Improve Left Ventricular Remodelling after Myocardial Infarction

ROS generated by IR injury post myocardial infarction may play a role in cellular signalling pathways leading to remodelling, hypertrophy, dilatation and dysfunction of the LV after myocardial infarction (225, 226). Allopurinol can improve LV remodelling in mice with extensive anterior myocardial infarction, reducing myocardial hypertrophy and interstitial fibrosis. In mice models, allopurinol reduced ROS production and XO expression as measured using electron spin resonance spectroscopy (225). Again using mice, a recent paper looked at the effects of XO inhibitors in mice with induced diabetic cardiomyopathy using streptozotocin. Interestingly XO activity in the myocardium, liver and serum of the diabetic mice was increased. Allopurinol decreased this XO activity compared with placebo over 10 weeks, which correlated with an improvement in systolic and diastolic function. Therefore a conclusion was drawn that cardiac function in these mice improved by decreasing OS with XO inhibitors in diabetic mice (227).

Based on these studies on animals Guan *et al* (2003) in Japan hypothesised allopurinol may have beneficial effects on free radical generation in humans and therefore LV function post primary coronary angioplasty (213). They gave 400mg of allopurinol orally just after admission (approximately 60 minutes before reperfusion). It should be noted the numbers were small (control group 20 patients and allopurinol group 18 patients) and they used measurement of urinary 8-epi-prostaglandin F2 α to assess free radical

production (213). This study added support to the argument that allopurinol inhibits the generation of oxygen derived radicals in IR injury in humans. They also found benefits in the recovery of LV function after primary coronary angioplasty for myocardial infarction (213).

h. Allopurinol Improves Endothelial Function in Various Cardiovascular Disorders

In order to see if allopurinol is beneficial in heart failure, Struthers *et al* (2002) performed a retrospective cohort observational study at the University of Dundee on 1760 patients with chronic heart failure (228). They found that if a patient receives 300mg or more of allopurinol per day, there was a significantly better mortality than those receiving less than 300mg over long periods. This highlights the need to ensure the correct dose of allopurinol is used in the investigation of the potential therapeutic effects of allopurinol. This is discussed further in the section that concerns the 'dose of allopurinol' question.

Following on from this study, George *et al* (2006) have shown the mechanism of action of allopurinol is that it improves endothelial function by profoundly reducing vascular OS and not by lowering uric acid (211). The cohorts of patients studied were chronic heart failure patients New York Heart Association Class II-III. Endothelial function was assessed by standard forearm venous occlusion plethysmography on a range of allopurinol 300mg or 600mg against placebo. There was a marked increase in endothelial

function over placebo with both doses and 600mg was much better than 300mg, discussed in further detail under the section 'dose of allopurinol'. In fact the reduction in urate on increasing allopurinol from 300mg to 600mg was much smaller (17%) than the added improvement in endothelial function (211). The main important finding from this study was that allopurinol abolished the vitamin C sensitive component of vascular OS. This implies that at a tissue level, allopurinol virtually eradicates OS since, after allopurinol, the anti-oxidant vitamin C is ineffective as there is no endogenous OS left to scavenge. Furthermore probenecid, which lowers urate, had no effect on endothelial function, implying that the mechanism by which allopurinol improves endothelial function is by reducing OS and not by lowering urate. Doehner *et al* (2002) also found allopurinol improved endothelial function in chronic heart failure (229). In that study allopurinol was given by intra-arterial infusion and orally (at 300mg once daily for one week). Endothelial function was measured by an improvement in peak blood flow using venous occlusion plethysmography both systemically and locally. Also of note was a decrease by 20% of allantoin, a marker of oxygen free radicals (229).

A study of 23 patients by Butler *et al* (2000) looked at endothelial function in a cohort of T2DM with mild hypertension (212). Diabetes is a condition characterised by high levels of OS, as discussed in chapter one. The dose of allopurinol used was 300mg for one month in this double blind control trial. Bilateral venous occlusion plethysmography was used to assess endothelial function. Allopurinol improved endothelial function in these patients (to near normal levels) but had no effect on endothelial function in matched control

subjects who did not have OS (212). To assess oxygen free radical activity, they used malondialdehyde levels, and this was significantly reduced in the allopurinol group (0.30 ± 0.04 versus $0.34 \pm 0.05 \mu\text{mol/L}$ for allopurinol versus placebo, $P=0.03$) (212).

It is thought that there is also increased OS in obstructive sleep apnoea resulting in endothelial dysfunction (230). Hence El Solh *et al* (2006) investigated the possible beneficial effects of allopurinol on endothelial function in this group of patients. Twelve patients were randomised to 300mg of allopurinol or placebo and endothelial function was measured using FMD. There was a significant increase in FMD (10.4 ± 3.2 versus $7.4 \pm 2.8\%$ for placebo) indicating another potential disease where allopurinol may improve endothelial function (230).

Guthikonda *et al* (2003) used allopurinol in cigarette smokers to see if it improved endothelial dysfunction (215). Cigarette smoking is thought to cause endothelial dysfunction through increasing OS (215). Interestingly 600mg allopurinol was given as a single dose on the day of the study and endothelial function was measured assessing forearm blood flow responses to acetylcholine and bradykinin. They found endothelial function was improved in this small number of patients and therefore that XOR may play a role in the endothelial dysfunction caused by cigarette smoking (215).

i. Allopurinol Improves Augmentation Index and Brain Natriuretic Peptide

In studies published from the University of Dundee, it has often been shown that allopurinol reduces Alx and BNP. In stroke patients, allopurinol reduced Alx from 26.08 \pm 3.31% to 20.15 \pm 2.23% compared with an increase in the placebo group from 23.57 \pm 3.13% to 27.64 \pm 3.44% (231). Allopurinol reduced BNP significantly in 50 heart failure patients (232). These findings are important, as already discussed previously, Alx reflects after load and BNP is raised in any condition putting pressure on the heart, suggesting allopurinol may therefore reduce intra-cardiac pressure, possibly by an effect in reducing LVM.

j. Effects of Allopurinol in Ischaemic Stroke

In ischaemic stroke allopurinol has shown potential in reducing complications as high uric acid levels have been shown to be associated with worse outcomes (233). Allopurinol has beneficial effects on inflammatory markers after ischaemic stroke, however allopurinol has had limited effect on cerebrovascular activity after stroke (234, 235). As previously discussed allopurinol may also have beneficial effects in stroke prevention due to in some part its effects in reducing LA size and reducing the burden of AF.

k. Effects of Allopurinol on Blood Pressure

Most studies to date have shown no significant effect of allopurinol on BP (211, 236). There has been one study that has shown allopurinol decreases BP in adolescents with newly diagnosed essential hypertension (237). The study design was cross-over of 30 patients over four weeks and the fall in systolic BP was modest (-6mmHg). A similar finding was seen when allopurinol was given to 48 hyper-uricaemic patients and compared to normo-uricaemic patients. Allopurinol had small improvements in BP over three months in the hyper-uricaemic patients but there was no effect on BP in the normo-uricaemic patients (238). Agarwal *et al* (2013) performed a systematic review looking at all the trials that assessed the efficacy of allopurinol on BP (239). A total of 10 clinical studies with 738 participants were included in the analysis. Compared with the control group, systolic BP decreased by 3.3 mmHg (95% CI 1.4-5.3 mm Hg; P=0.001). When the analysis was restricted to the higher-quality randomised controlled trials, similar changes BP was found with a fall in SBP by 3.3 mm Hg (95% CI, 0.8-5.8 mm Hg; P<0.001). There is an association between high uric acid and raised BP (240). It may be that allopurinol has small effects on BP in specific sub-sets of patients with high uric acid.

l. Allopurinol May Regress Left Ventricular Hypertrophy

Cingolani *et al* (2006) studied oxypurinol for four weeks in patients with heart failure (241). This was a short period of time, but there was a non-statistically

significant trend towards a reduction in LVM. This small, short study was the first to suggest that XO inhibitors may regress LVH.

Very recently, two studies have been published showing that allopurinol can regress LVH in two different cohorts, patients with renal dysfunction and patients with chronic stable angina (236, 242). Kao *et al* (2011) studied 67 patients with chronic kidney disease and gave 300mg once daily allopurinol over nine months (236). LVM fell by $1.42 \pm 4.67\text{g/m}^2$ in the active group and rose by $1.28 \pm 4.45\text{g/m}^2$ in the placebo group. Rekhraj *et al* (2013) found very similar results in 66 patients with chronic stable angina. Allopurinol was given at a higher dose in this study, 600mg per day. LVM fell by $2.2 \pm 2.78\text{g/m}^2$ in the active group and rose by $0.53 \pm 2.5\text{g/m}^2$ in the placebo group. In both of these studies the fall in LVM is modest albeit significant, which makes it very possible that it could do the same in patients with diabetes.

m. Dosing of Allopurinol

Until recently only one study used 600mg once daily of allopurinol and this was by Guthikonda *et al* (2003) on heavy smokers (215). At this dose, allopurinol rapidly reduced smoking induced endothelial dysfunction. Most previous research using allopurinol had been with 300mg once daily, some of which have been discussed above e.g. Farquharson *et al* (2002) gave 300mg to chronic heart failure patients New York Heart Association class II – III for one month versus placebo. They found that 300mg allopurinol improved endothelial function in this small cohort significantly using the forearm

plethysmography way on measuring endothelial function (214). George *et al* (2006) then looked carefully at the dose response relationship between allopurinol and endothelial function. Again this study looked at chronic heart failure patients New York Heart Association class II – III (30 patients). George *et al* (2006) found increasing the dose of allopurinol from 300mg to 600mg improved endothelial function by 52%. Overall allopurinol 600mg improved endothelial function by 143% compared to placebo. The interesting conclusion is that previous studies using 300mg allopurinol had actually used a suboptimal dose of allopurinol. Allopurinol was well tolerated even in this cohort where 600mg per day was used. This may also help explain Struthers *et al* (2002) findings that if a patient receives 300mg or more of allopurinol per day there was a significantly better mortality than those receiving less than 300mg over long periods (228).

6. **Aims**

The aims of the study are based on the discussion above. In summary, diabetes is still a major cause of CV morbidity and mortality despite recent advances in treatments. Patients with diabetes are particularly prone to LVH, and LVH is an important, often forgotten, CV risk factor. Despite aggressive treatments for LVH with blockade of the RAS and lowering of BP, LVH still remains a problem in diabetic patients. Therefore, a treatment is needed over and above traditional treatments and we propose allopurinol may be of benefit in these patients. There are three main reasons to suggest allopurinol may regress LVH in T2DM: improvement in endothelial function, reduction in Alx and the recent findings that allopurinol regresses LVH in kidney disease and chronic stable angina. The endpoints of the study are:

1. The primary endpoint is to assess if allopurinol regresses LVH in patients with T2DM and BP below 150/90. LVM will be assessed by CMR.
2. The secondary endpoints are to assess if allopurinol improves endothelial function and Alx in diabetic patients. Endothelial function will be assessed using FMD and Alx with Sphygmocor technology.

CHAPTER TWO

METHODS

1. Approvals and Trial Registration

Ethical Approval

The clinical trial was approved by the Tayside Research Ethics Committee A, reference number 09/S0501/3.

Medicines and Health Regulatory Approval (MHRA)

The clinical trial and use of allopurinol and placebo was approved by the MHRA, EudraCT number 2008-008485-12.

Trial Registration

This study is registered as UK Clinical Research Network Number (UKCRN) 8766.

2. Study Design

The study design was a single centre randomised, double blind, placebo-controlled trial with nine months follow up. The active drug was 600mg per day of allopurinol. All participants provided written informed consent to participate in this study.

66 patients were recruited, 33 patients randomised to receive allopurinol and 33 randomised to receive placebo. Patients were allocated a computer generated treatment code (patient and investigators blinded). Patients continued their other medications including anti-hypertensives and insulin.

a. Study participants

Patients were identified from databases of out-patients. Patients were identified by the Scottish Diabetes Research Network, the Health Informatics Centre or the Scottish Primary Care Research Network if they had T2DM. Essentially any patient with T2DM was screened for entry.

Patients had to have T2DM and an average office BP of less than 150/90 measured over three readings. Echo was performed to assess for echo LVH. LVH on echo was defined by the American Society of Echocardiography criteria of LVM index of greater than 115g/m^2 for men and greater than 95g/m^2 for women (using the average of three measurements of LVM by one trained operator (BRS)) (243).

b. Exclusion Criteria

Exclusion criteria included: gout, patients currently prescribed allopurinol, previous adverse reaction to allopurinol, eGFR less than 60ml/min/1.73m², conditions that exclude CMR, LV ejection fraction less than 45%, cancer or other life threatening illness, pregnancy or breast feeding and being unable to provide consent.

c. Allopurinol: Dosing and Side Effects

The dose of allopurinol used was 600mg per day in two divided doses of 300mg.

Allopurinol is a drug that has been used for over 40 years. According to the BNF it can be given in doses up to 1000mg/day. Caution should be used in hepatic and renal impairment. Pregnancy and breast feeding is a contra-indication for use. Rashes and gastrointestinal disorders are the commonest side effects. Hypersensitivity reactions occur rarely and include exfoliation, fever, lymphadenopathy, arthralgia, and eosinophilia resembling Stevens-Johnson or Lyell's syndrome. The BNF lists malaise, headache, vertigo, drowsiness, visual and taste disturbances, hypertension, alopecia, hepatotoxicity, paraesthesia and neuropathy, gynaecomastia, blood disorders (including leucopenia, thrombocytopenia, haemolytic anaemia and aplastic anaemia) as being very rare.

d. Study visits and Drug Titration

After recruitment, patients attended for six visits over a nine-month period. An initial dose of allopurinol 100mg per day or placebo was dispensed and this was increased to 300mg per day or placebo after two weeks. The dose was further increased to 600mg per day or placebo at four weeks and continued for the duration of the trial. The study visits are outlined in Figure 2.2.1. Office BP was measured for all the patients at each visit and nine randomly selected patients had a 24-hour ambulatory BP monitor at baseline and final visit. Availability of ambulatory monitors was limited which is why 24-hour BP was only done in a random subset.

The final dose of allopurinol was decided upon when there were no reported side effects or change in full blood count, renal and liver function. Patients were told to inform their General Practitioner of any side effects and the Chief Investigator. Contact details of the chief investigator were given to both patient and GP.

The criteria for stopping allopurinol was if they had any of the following side effects: rashes, gastrointestinal disorders, hypersensitivity reactions, malaise, headache, vertigo, drowsiness, visual and taste disturbances, hypertension, alopecia, hepatotoxicity, paraesthesia and neuropathy, gynaecomastia, blood disorders (including leucopenia, thrombocytopenia, haemolytic anaemia and aplastic anaemia). If there was an increase in creatinine by 26 $\mu\text{mol/L}$ from

baseline. Also if there was an increase in ALT/AST (liver function tests) by three times the upper limit of normal.

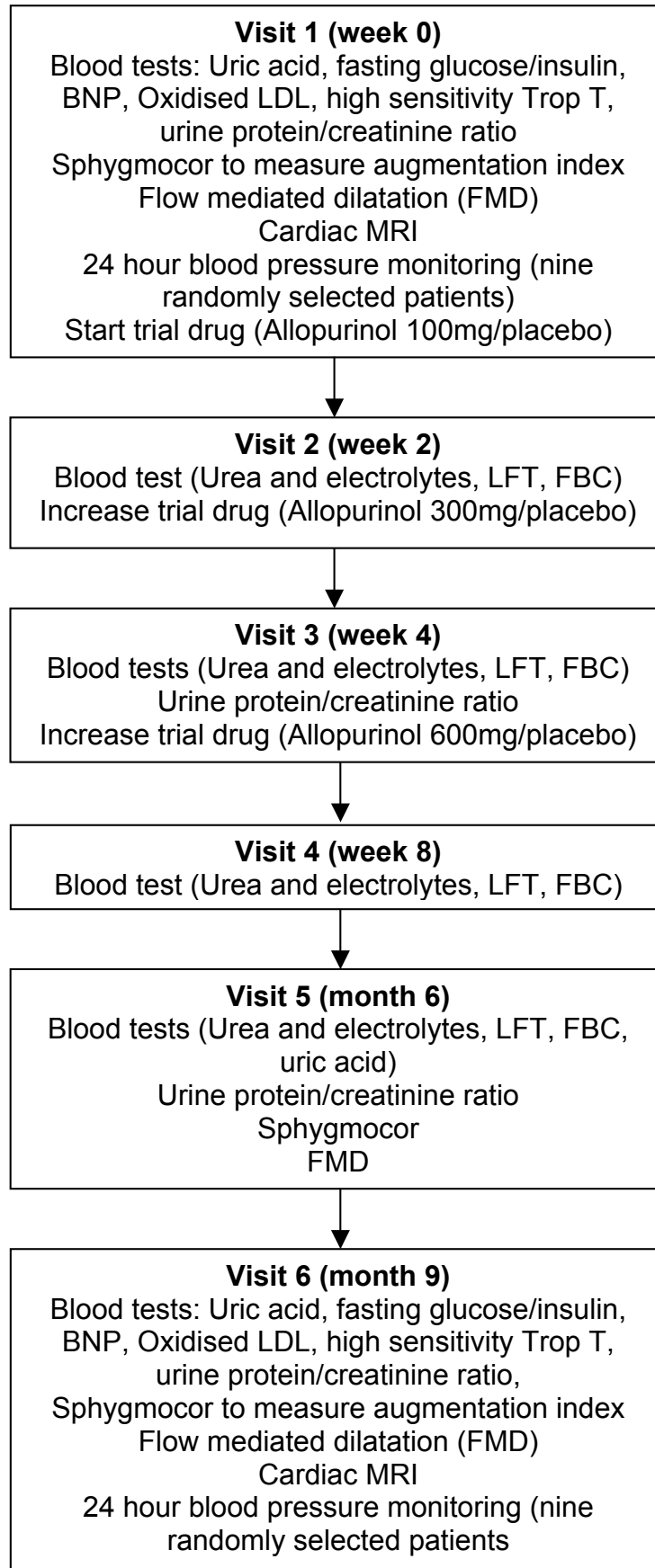


Figure 2.2.1. Outline of Study Visits.

e. Cardiac Magnetic Resonance Imaging

CMR was performed at baseline and at nine months only using a 3T Magnetom Trio scanner (Siemens, Erlangen, Germany). The scanner uses a body coil for RF transmission, and a combination of body array and spine matrix coils for RF detection. Short axis images from the atrio-ventricular ring to the LV apex were acquired using a 2D ECG-gated breath-hold segmented CINE TrueFISP sequence with retrospective gating. The imaging parameters included repetition time TR 3.4ms, Echo time of 1.5ms and flip angle 50°. Each slice was 6mm thick (with 4mm gap between adjacent slices). Two slices were acquired per breath hold of less than 15 seconds and scan time was minimized using parallel imaging “GRAPPA” (factor two).

Quantitative measurement of LVM, ejection fraction (EF), end-diastolic volume (EDV), end-systolic volume (ESV) and stroke volume (SV) were derived by ‘region of interest’ contours placed around endocardial and epicardial LV borders. This was done on all CMR image slices at end-diastole and end-systole that were identified to contain 50% or more full-thickness myocardium. All CMR images were analysed using commercial software (‘Argus’, Siemens Multi-modality Work Platform, version VB 15). An independent MRI Physicist (Steve Gandy) analysed all images in fully ‘blinded’ fashion. Papillary muscles were included in the LVM if the muscle structure was indistinguishable from the myocardial wall, but otherwise assigned to the LV blood pool. Contouring was performed twice for every dataset to ensure that repeatability was consistently <5%. Figure 2.2.2 shows

an example of CMR images, highlighting typical endocardial and epicardial border region-of-interest contouring of a set of images from the study. In this example, a total of nine slices were acquired from the base of the LV myocardium (image 1-top left) to the apex (image 9-bottom right).

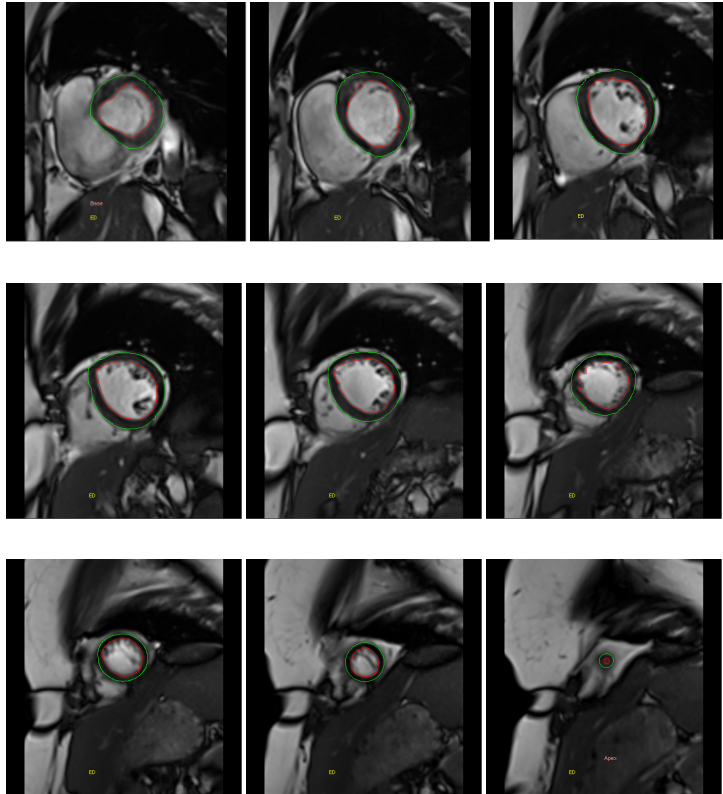


Figure 2.2.2 Typical endocardial and epicardial border region-of-interest contouring of a set of images from the study.

f. Flow-mediated Dilatation

Endothelial function was assessed by measuring FMD of the brachial artery. FMD was measured using a Sequoia 512 (Siemens, Camberley, UK) ultrasound machine with a 8 MHz linear array probe. FMD was performed at baseline, six months and nine months, shown in Figure 2.2.1. The response

to hyperaemia and endothelial independent dilatation was performed as per the International Brachial Artery Reactivity Task Force guidelines and has been performed regularly at our institute (211, 244, 245). The brachial artery image was located and a baseline measurement captured over one minute. Firstly the artery was assessed for the response to hyperaemia by inflating the cuff distal to the elbow to 200mmHg for five minutes. The cuff was then rapidly deflated and the reactive hyperaemia was recorded for two minutes. A 10-minute rest period was included before assessment of endothelial independent dilatation. This was done by assessing the response of brachial artery size to two puffs of sublingual glyceryl trinitrate spray. FMD was the percentage change in average diameter achieved after cuff deflation relative to the baseline average brachial artery diameter. The FMD was analysed using Vascular Research Tools software (Medical Imaging Applications LLC, Coralville, IA, USA). The acquisition and analyses of the FMD images was performed by a single trained investigator (BRS), who was blinded to the allocated treatment.

g. Applanation Tonometry

Pulse wave analysis (PWA) and pulse wave velocity (PWV) were measured at baseline, six months and nine months by a single trained investigator (BRS) who was blinded to the allocated treatment. Analysis was performed using an Sphygmocor (AtCor, Sydney, Australia) machine using a high fidelity micromanometer. Prior to taking measurements patients were lay supine for 10 minutes. Firstly, for PWA, the radial artery was used to acquire peripheral

pressure waveforms (radial artery waveform) by applanation tonometry. The radial artery waveform was used to generate the central aortic pressure using the Sphygmocor manufactures validated calculation factors. Alx was calculated as the difference between the first and second systolic peaks from the calculated aortic pressure wave. Alx is expressed as a percentage of the pulse pressure normalised for a heart rate of 75 beats per minute. Secondly for PWV, radial and carotid arterial waveforms were obtained with ECG gating so the velocity could be calculated measuring the time interval between R-wave and the pulse waveforms. This was performed at the radial and carotid arteries separately and the distance between the two sites measured.

h. Laboratory Methods

i. Biochemistry and Haematology Tests

Blood samples for haemoglobin, urea and electrolytes, liver function tests, glucose, HBA1c, urine PCR and uric acid were all analysed locally at the laboratories in Ninewells Hospital, Dundee. The samples were collected using standard vacutainer tubes and the samples stored at room temperature before immediate transfer to our laboratory.

ii. Brain Natriuretic Peptide

BNP was collected in a 5ml. EDTA blood tube. Blood was centrifuged then the plasma was decanted into an aliquot that was stored at -70⁰C. The assay

was a radio-immunoassay using Bachem UK equipment. The inter-observer variability was 26% and the intra-observer variability was 14%.

iii. Uric Acid

Uric acid was not analysed until the end of the trial as changes in uric acid may have given clues to the drug prescribed during the trial. Blood was centrifuged then the plasma was decanted into an aliquot that was stored at -20°C. The assay was a colorimetric assay using Alpha (sentinel kit) equipment. The inter-observer variability was 5% and the intra-observer variability was 5.4%.

iv. Insulin

Blood was centrifuged then the plasma was decanted into an aliquot that was stored at -20°C. The assay was a radio-immunoassay using Diasorin UK equipment. The inter-observer variability was 4.40%. The normal range quoted by the manufacturer is <5.25uU/L.

v. Oxidised LDL

Oxidised LDL was collected in a 5ml. EDTA blood tube. Blood was centrifuged then the plasma was decanted into an aliquot that was stored at -70°C. The assay was an ELISA assay using Mercodia Diagenics, Sweden

equipment. The inter-observer variability was 15% and the intra-observer variability was 8.6%.

i. Drug Manufacture and Randomisation

The study trial drugs were manufactured by Pharmacy Production Unit, St. Thomas' Hospital, London, UK. The blinding and randomisation was conducted by the Clinical Trials Pharmacy Department, Ninewells Hospital, Dundee. The un-blinding code was held by the pharmacy department. The trial was un-blinded when the database was locked and all data had been analysed in a group only fashion.

J. Statistical Analysis

66 patients were required to achieve 90% power to detect a clinically significant change in LV mass based on previous studies (246) and 80% power to detect a clinically significant change in FMD at a significance level of $p < 0.05$, allowing for a 20% dropout rate. Using published data from Grothues *et al* (2002), 60 completed patients were anticipated to have 90% power to detect a five gram difference in LVM between active and placebo which is a figure similar to the difference between the two treatments in the echo sub-study of the LIFE study (see below) (247).

Data for continuous variables are presented as mean (SD) for normally distributed data and median and inter-quartile range for non-normally

distributed data. Categorical data are expressed as numbers (%). Comparison between continuous variables were analysed using the Student t-test or Mann-Whitney U test whilst categorical variables were analysed using χ^2 test. Correlation analysis was performed for changes in LVM and LVMI. A multivariate ANOVA using important covariates was also performed for baseline BP, change in BP and prescription of ACE inhibitor or ARBs. These variables were chosen, as they may have been confounding factors in allopurinol's effects. Change in LVM was not incorporated into the multivariate analysis as this was accounted for in the analysis of LVM indexed to BSA.

All statistical analyses were undertaken using SPSS version 18.0 (SPSS, Chicago, IL). A two-sided p value < 0.05 was considered statistically significant.

CHAPTER THREE **RESULTS**

1. Study Recruitment

In total, 66 patients were recruited, and this was the target number. 33 patients were recruited to the allopurinol arm and 33 patients to the placebo arm of the trial. Recruitment is described graphically in the Consort diagram, Figure 3.1.1.

Seven participants withdrew from the study (three from the placebo group and four from the active group), Figure 3.1.1. A further four participants were unable to undergo CMR (one from the placebo group and three from the active group). Therefore 56 participants completed the CMR parts of the trial and 59 completed all other aspects. The reasons for withdrawing from the trial included: side effects $n = 3$, cancer $n = 1$, pacemaker insertion $n=1$ and patient preference $n = 2$. One patient developed a rash thought to be due to allopurinol. One patient felt nauseated but turned out to have been on placebo. Another patient felt more short of breath and although it was felt not a classical side effect of allopurinol the patient was withdrawn. During the study period, one patient required insertion of a pacemaker that was felt to invalidate the trial due inability to have the final MRI scan and the possible haemodynamic effect on endothelial function that may have invalidated further FMD. The requirement for a pacemaker was not thought to be related to allopurinol. Of the patients unable to undergo CMR the reasons where: obesity $n = 1$ and claustrophobia $n = 3$. Therefore the percentage of adverse

events in this study was 4.5%. Of the 162 patients screened at entry, 96 patients failed screening due to: raised BP n= 24, renal function n= 9, no LVH on echocardiogram n = 77, patient preference n = 7 and valvular disease n = 2 (there could, of course, be more than one reason for failing screening).

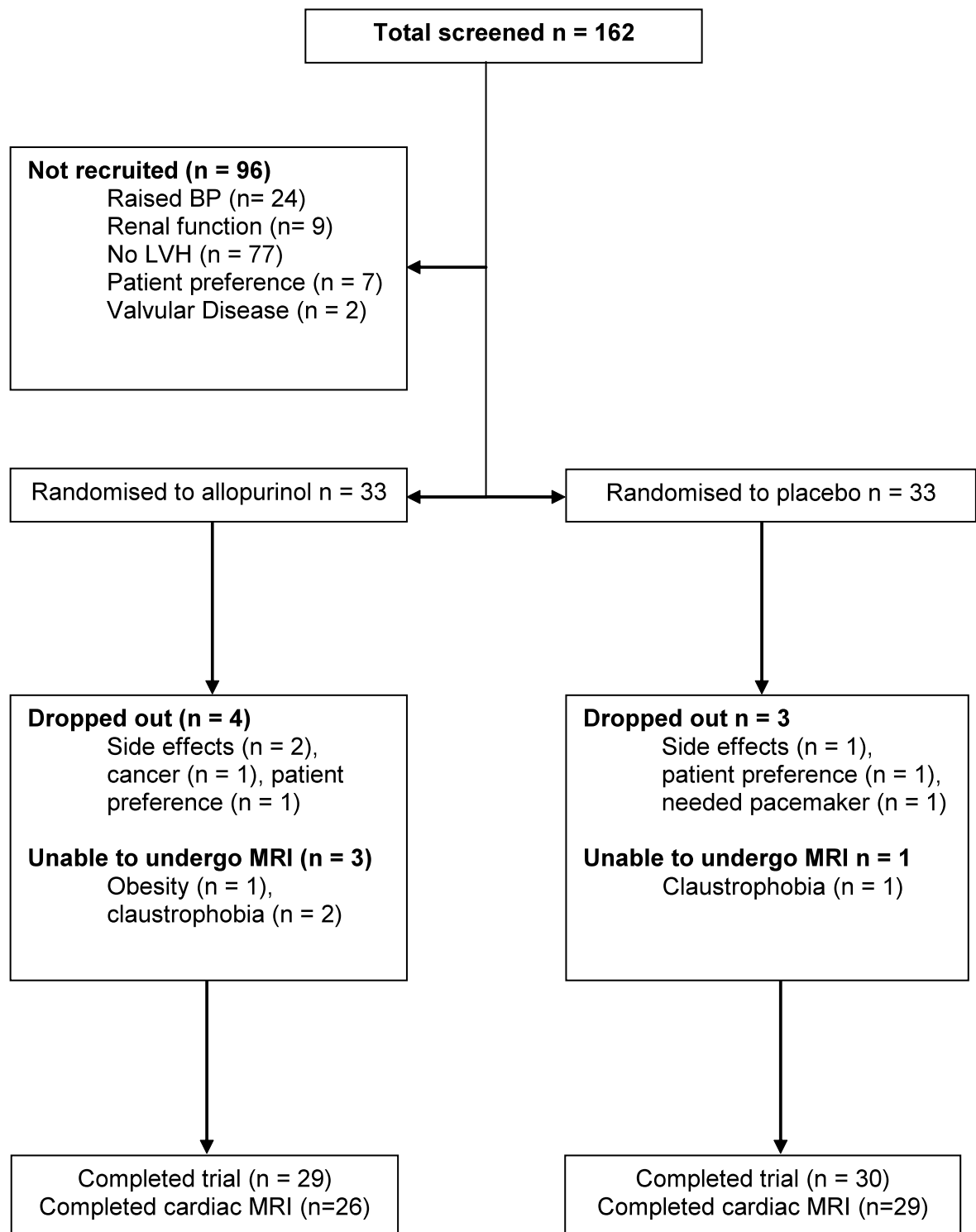


Figure 3.1.1. Study recruitment CONSORT diagram.

2. Baseline Characteristics

Baseline characteristics are shown in Table 3.2.1. There were no significant differences between the groups for baseline characteristics other than for body mass index (BMI). Importantly this included LVM, BP, uric acid and medications including ACE inhibitors. The average duration of diabetes was nine years before entry into the trial. Patients were well treated with modern risk factor modification namely ACE inhibitors (49%), ARB (21%), beta-blockers (23%) and statins (85%).

| Variable | All patients | Placebo | Allopurinol | P value |
|--|----------------|----------------|----------------|---------|
| Total patients | N = 59 | N = 30 | N= 29 | |
| Did not have MRI | 4 | 1 | 3 | |
| Completed MRI | 56 | 29 | 26 | |
| Completed FMD | 59 | 30 | 29 | |
| Mean Age (years) | 64.63 ± 8.79 | 66.03 ± 8.86 | 63.17 ± 8.64 | 0.214 |
| Male | 36 (61.02%) | 21 (70.00%) | 16 (55.17%) | 0.101 |
| BMI baseline | 32.59 ± 4.78 | 31.12 ± 3.93 | 34.13 ± 5.14 | 0.014 |
| 24 hour SBP baseline | 121.67 ± 11.55 | 122.25 ± 11.59 | 121.20 ± 12.87 | 0.903 |
| 24 hour DBP baseline | 66.22 ± 3.31 | 68.25 ± 2.75 | 64.60 ± 2.97 | 0.101 |
| Office SBP baseline | 139.51 ± 11.33 | 141.63 ± 11.03 | 137.31 ± 11.41 | 0.144 |
| Office DBP baseline | 77.66 ± 8.69 | 76.70 ± 10.18 | 78.66 ± 6.87 | 0.392 |
| Echo LV mass index (g/m ²) | 126.18 ± 19.04 | 126.39 ± 17.46 | 125.97 ± 20.88 | 0.933 |
| Absolute MRI LV mass (g) (n=56) | 122.60 ± 28.32 | 119.92 ± 27.89 | 125.59 ± 29.36 | 0.464 |
| MRI LV mass index (g/m ²) (n=56) | 60.67 ± 9.78 | 60.16 ± 10.11 | 61.23 ± 9.56 | 0.691 |
| Hypertension | 53 (89.83%) | 27 (90.00%) | 26 (89.66%) | 0.534 |
| IHD | 6 (10.17%) | 3 (10%) | 3 (10.34%) | 0.534 |
| Stroke | 6 (10.67%) | 5 (16.67%) | 1 (3.45%) | 0.026 |
| Raised cholesterol | 54 (91.52%) | 27 (90.00%) | 27 (93.10%) | 0.056 |
| Smoker | 8 (13.56%) | 6 (20.00%) | 2 (6.90%) | 0.454 |
| Ex-smoker | 26 (44.07%) | 11 (36.66%) | 15 (51.72%) | 0.518 |
| Never smoked | 25 (42.37%) | 13 (43.33%) | 12 (41.38%) | 0.638 |
| Duration of diabetes (years) | 9.46 ± 6.02 | 8.97 ± 5.36 | 9.97 ± 6.68 | 0.528 |
| ACE inhibitor | 29 (49.15%) | 13 (43.33%) | 16 (55.17%) | 0.363 |
| ARB | 16 (21.73%) | 8 (26.67%) | 8 (27.59%) | 0.937 |
| CCB | 16 (27.12%) | 11 (36.67%) | 5 (17.24%) | 0.093 |
| Thiazide diuretic | 13 (22.03%) | 8 (26.67%) | 5 (17.24%) | 0.383 |
| Furosemide | 4 (6.78%) | 3 (10.00%) | 1 (3.45%) | 0.317 |
| Beta-blocker | 14 (23.73%) | 7 (23.33%) | 7 (24.14%) | 0.942 |
| Metformin | 45 (76.27%) | 20 (66.67%) | 25 (86.21%) | 0.078 |
| Gliclazide | 15 (24.42%) | 7 (23.33%) | 8 (27.58%) | 0.708 |
| TZD | 7 (11.86%) | 3 (10.00%) | 4 (13.79%) | 0.652 |
| Exenatide | 2 (3.39%) | 0 (0.00%) | 2 (6.90%) | 0.143 |
| Insulin | 9 (15.25%) | 4 (13.33%) | 5 (17.24%) | 0.676 |
| Aspirin | 28 (47.46%) | 15 (50.00%) | 13 (44.53%) | 0.691 |
| Clopidogrel | 2 (3.39%) | 0 (0.00%) | 2 (6.90%) | 0.143 |
| Statin | 50 (84.75%) | 25 (83.33%) | 28 (86.21%) | 0.759 |
| Haemoglobin (g/L) | 13.73 ± 1.43 | 14.06 ± 1.27 | 13.40 ± 1.53 | 0.076 |
| eGFR (ml/min/1.73 ²) | 86.31 ± 13.75 | 83.21 ± 10.38 | 89.52 ± 16.09 | 0.078 |
| Creatinine (μm/L) | 76.88 ± 12.08 | 80.00 ± 11.16 | 73.66 ± 12.33 | 0.043 |
| Fasting glucose (mmol/L) | 6.62 ± 1.96 | 6.14 ± 1.66 | 7.11 ± 2.15 | 0.056 |
| Fasting insulin (uU/L) | 32.16 ± 25.34 | 34.37 ± 27.74 | 29.88 ± 22.87 | 0.501 |
| HBA1c (%) | 7.25 ± 0.94 | 7.12 ± 0.83 | 7.39 ± 1.04 | 0.274 |

| | | | | |
|---------------------|----------------|----------------|----------------|-------|
| Urine PCR (mg/mmol) | 13.05 ± 8.26 | 14.13 ± 10.51 | 11.93 ± 4.93 | 0.310 |
| BNP (pg/ml) | 29.01 ± 25.81 | 30.01 ± 22.74 | 27.97 ± 29.03 | 0.764 |
| Uric acid (mmol/L) | 0.54 ± 0.13 | 0.54 ± 0.10 | 0.55 ± 0.15 | 0.613 |
| PIINP (n=23) (ug/l) | 4.37 ± 1.05 | 4.56 ± 0.94 | 4.16 ± 1.16 | 0.379 |
| HS TropT (ng/L) | 7.46 ± 4.85 | 7.90 ± 5.59 | 7.01 ± 3.99 | 0.486 |
| Oxidised LDL (U/L) | 28.51 ± 9.90 | 27.58 ± 10.07 | 29.48 ± 9.81 | 0.467 |
| | | | | |
| EF (%) | 75.15 ± 5.317 | 75.21 ± 4.66 | 75.08 ± 5.78 | 0.927 |
| EDV (mls) | 123.52 ± 35.94 | 118.36 ± 36.41 | 129.28 ± 35.23 | 0.264 |
| ESV (mls) | 31.17 ± 13.01 | 29.80 ± 12.00 | 32.71 ± 14.12 | 0.412 |
| SV (mls) | 92.36 ± 25.42 | 88.57 ± 26.06 | 96.58 ± 24.50 | 0.247 |
| CO (mls) | 6.41 ± 1.60 | 6.18 ± 1.70 | 6.66 ± 1.47 | 0.265 |
| | | | | |
| FMD (%) | 4.11 ± 0.08 | 4.16 ± 0.59 | 4.07 ± 0.64 | 0.578 |
| Alx (%) | 11.09 ± 10.07 | 10.47 ± 10.78 | 11.76 ± 9.40 | 0.629 |
| PWV (m/s) | 7.07 ± 1.14 | 6.94 ± 1.03 | 7.19 ± 1.24 | 0.468 |

Table 3.2.1. Baseline characteristics.

3. Changes in Measured Parameters

The main finding from this study is that allopurinol significantly reduced LVM over the nine-month study period both for absolute LVM and LVMI. Change in LVM was $-2.65 \pm 5.91\text{g}$ and $+1.21 \pm 5.10\text{g}$ ($p=0.012$) for the allopurinol group and placebo group respectively. Change in LVMI was $-1.32 \pm 2.84\text{g/m}^2$ and $+0.65 \pm 3.07\text{g/m}^2$ ($p=0.017$) for the allopurinol group and placebo group respectively. The results are detailed further in Table 3.3.1 and in Figure 3.3.1. Multivariate analysis was performed using co-variates of baseline BP, change in BP and prescription of ACE inhibitor or ARBs that showed $p=0.013$ for LVM and $p=0.021$ for LVMI, Table 3.3.2. It is quite common for LVM to increase with ageing in the placebo group of MRI studies, as we saw here and have seen before (248).

| | Placebo | Allopurinol | P value |
|--------------------------------|------------------|------------------|---------|
| Change LVM (g) | $+1.21 \pm 5.10$ | -2.65 ± 5.91 | 0.012 |
| Change LVMI (g/m^2) | $+0.65 \pm 3.07$ | -1.32 ± 2.84 | 0.017 |
| | | | |
| Change in EF (mls) | 0.08 ± 7.41 | 1.46 ± 6.10 | 0.458 |
| Change in EDV (mls) | 4.45 ± 39.60 | 6.12 ± 40.57 | 0.878 |
| Change in ESV (mls) | 2.44 ± 21.84 | 0.20 ± 14.60 | 0.660 |
| Change in SV (mls) | 2.01 ± 24.83 | 5.89 ± 28.88 | 0.595 |
| Change in CO (L/min) | -0.75 ± 1.61 | 0.67 ± 1.45 | 0.081 |

Table 3.3.1: Changes in MRI parameters (Over 9 month study period)

Tests of Between-Subjects Effects

| Source | Dependent Variable | Type III Sum of Squares | df | Mean Square | F | Sig. |
|-----------------|--------------------|-------------------------|----|-------------|-------|------|
| Corrected Model | LVM | 399.779 ^a | 4 | 99.945 | 3.554 | .013 |
| | — LVMI | 105.239 ^b | 4 | 26.310 | 3.191 | .021 |
| Intercept | LVM | 1.870 | 1 | 1.870 | .067 | .798 |
| | — LVMI | 2.384 | 1 | 2.384 | .289 | .593 |
| SBP9 | LVM | 58.782 | 1 | 58.782 | 2.091 | .154 |
| | — LVMI | 10.911 | 1 | 10.911 | 1.323 | .255 |
| ACEI | LVM | 1.018 | 1 | 1.018 | .036 | .850 |
| | — LVMI | 5.832 | 1 | 5.832 | .707 | .404 |
| ARB | LVM | 118.950 | 1 | 118.950 | 4.230 | .045 |
| | — LVMI | 14.585 | 1 | 14.585 | 1.769 | .190 |
| Drugchange | LVM | 159.878 | 1 | 159.878 | 5.686 | .021 |
| | — LVMI | 48.516 | 1 | 48.516 | 5.884 | .019 |
| Error | LVM | 1405.900 | 50 | 28.118 | | |
| | — LVMI | 412.264 | 50 | 8.245 | | |
| Total | LVM | 1826.316 | 55 | | | |
| | — LVMI | 521.826 | 55 | | | |
| Corrected Total | LVM | 1805.680 | 54 | | | |
| | — LVMI | 517.503 | 54 | | | |

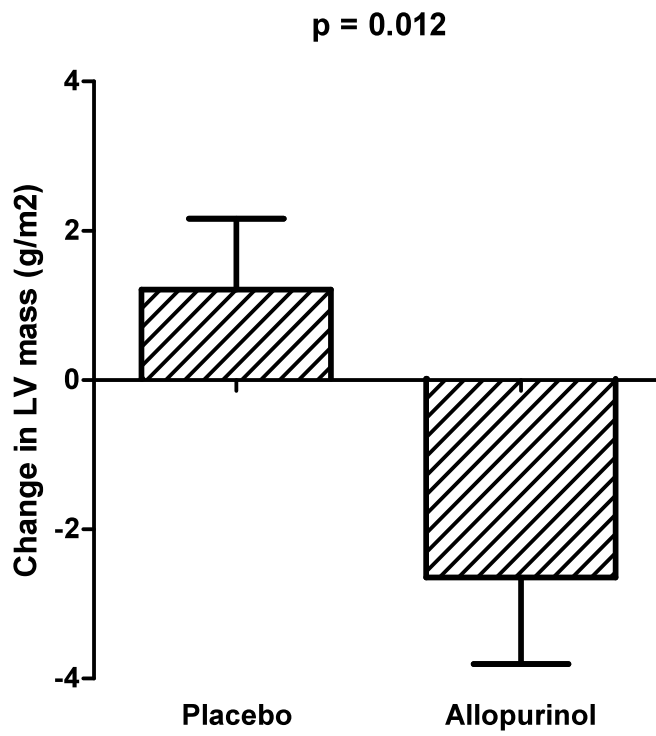
a. R Squared = .221 (Adjusted R Squared = .159)

b. R Squared = .203 (Adjusted R Squared = .140)

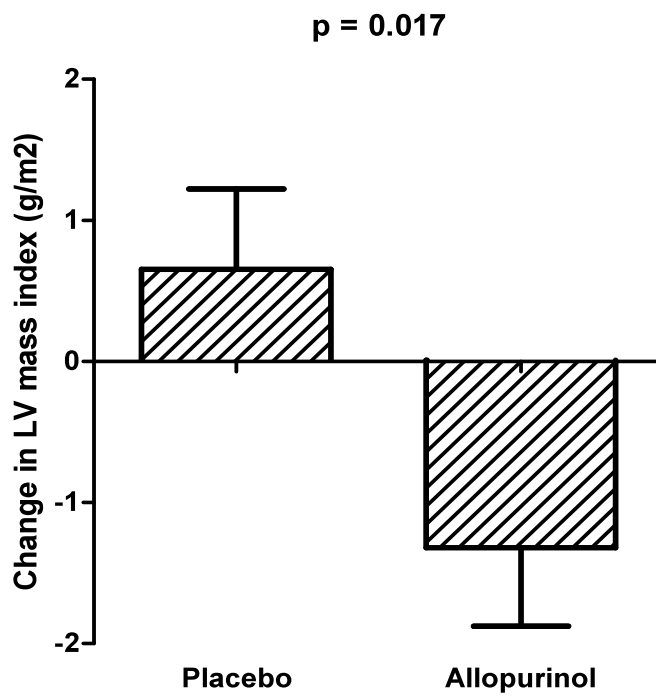
Table 3.3.2: Output from multivariate analysis using co-variates of baseline

BP, change in BP and prescription of ACE inhibitor or ARBs

(1)*



(2)*



*Standard Deviation

Figure 3.3.1 Diagrams of changes in (1) LVM and (2) LVMI

For the other parameters measured on cardiac MRI there was no significant changes in EF, EDV, ESV, SV and cardiac output (CO), shown in Table 3.3.1.

There was no change in BNP, oxidised low-density lipoprotein (LDL) or high sensitivity troponin T (HS Troponin T) over the study period, Table 3.3.3.

There was as expected a significant reduction in uric acid levels in the allopurinol group. Allopurinol was safe and had no affect on urine protein creatinine ratio (PCR) or eGFR. There was no difference in office or 24 hour BP over time, Table 3.3.4. No significant change was seen in both FMD and Alx as shown in Table 3.3.4.

| | Placebo | Allopurinol | P value |
|-----------------------------------|----------------|--------------------|----------------|
| Haemoglobin (g/L) | -1.50 ± 3.19 | -1.51 ± 2.99 | 0.993 |
| Creatinine (µm/L) | -7.00 ± 13.88 | -7.67 ± 7.39 | 0.910 |
| eGFR (ml/min//1.73 ²) | 6.57 ± 11.75 | 8.78 ± 10.00 | 0.441 |
| Fasting glucose (mmol/L) | 0.84 ± 4.05 | 0.41 ± 3.64 | 0.668 |
| Fasting insulin (uU/L) | -6.41 ± 19.26 | -1.64 ± 15.96 | 0.306 |
| HBA1c (%) | -0.063 ± 0.47 | -0.43 ± 1.80 | 0.279 |
| Urine PCR (mg/mmol) | 2.67 ± 12.76 | 5.03 ± 20.13 | 0.590 |
| BNP (pg/ml) | -1.49 ± 20.22 | -3.16 ± 20.36 | 0.754 |
| Uric acid (6 months) (mmol/L) | -0.019 ± 0.12 | -0.27 ± 0.17 | <0.001 |
| Uric acid (9 months) (mmol/L) | -0.01 ± 0.06 | -0.25 ± 0.18 | <0.001 |
| HS TropT (ng/L) | 0.34 ± 2.98 | 0.35 ± 2.25 | 0.656 |
| Oxidised LDL (U/L) | -0.11 ± 7.69 | 0.74 ± 8.80 | 0.692 |

Table 3.3.3. Change in blood parameters (Over 9 month study period)

| | Placebo | Allopurinol | P value |
|-------------------------------|----------------|--------------------|----------------|
| Office SBP (6 months) | -3.17 ± 17.08 | -1.69 ± 11.31 | 0.698 |
| Office SBP (9 months) | -6.50 ± 28.67 | 0.52 ± 14.82 | 0.245 |
| Office DBP (6 months) | -4.10 ± 14.13 | -8.55 ± 6.88 | 0.132 |
| Office DBP (9 months) | -4.53 ± 13.25 | -7.17 ± 8.44 | 0.367 |
| 24hr SBP (9 months) | -1.00 ± 0.61 | 0.41 ± 1.72 | 0.129 |
| | | | |
| FMD hyperaemia (6 months) (%) | -0.53 ± 2.93 | -0.079 ± 4.21 | 0.602 |
| FMD hyperaemia (9 months) (%) | 0.48 ± 4.38 | 1.00 ± 6.95 | 0.733 |
| FMD GTN (6 months) (%) | 1.42 ± 5.43 | 0.11 ± 1.21 | 0.405 |
| FMD GTN (9 months) (%) | 1.10 ± 4.84 | 0.84 ± 5.95 | 0.856 |
| | | | |
| Alx (6 months) (%) | -1.88 ± 9.46 | -1.51 ± 11.54 | 0.890 |
| Alx (9 months) (%) | -1.17 ± 12.67 | -1.36 ± 9.58 | 0.948 |
| | | | |
| PWV (6 months) (m/s) | -0.32 ± 1.75 | -0.87 ± 2.19 | 0.288 |
| PWV (9 months) (m/s) | -0.71 ± 2.65 | -0.70 ± 2.22 | 0.987 |

Table 3.3.4. Change in parameters of endothelial function and blood pressure

4. Changes in Measured Parameters for Above and Below Median LVM and LVMI

In order to assess the differing affects of having high or low LVM and LVMI at baseline as assessed by CMR, an analysis was made of measured parameters for each sub-group. The effect of LVM regression was more marked if the baseline LVM and LVMI was higher at baseline, as shown in Tables 3.4.1 and 3.4.2. For the above median baseline LVM sub-group change in LVM was $-4.03 \pm 6.77\text{g}$ and $+2.67 \pm 4.87\text{g}$ ($p=0.006$) for the allopurinol group and placebo group respectively. Change in LVMI in the above median LVM group was $-1.93 \pm 3.09\text{g/m}^2$ and $+1.32 \pm 2.13\text{g/m}^2$ ($p=0.004$) for the allopurinol group and placebo group respectively. For the above median baseline LVMI sub-group change in LVM was $-4.03 \pm 6.77\text{g}$ and $+1.42 \pm 6.77\text{g}$ ($p=0.025$) for the allopurinol group and placebo group respectively. Change in LVMI in the above median LVMI group was $-1.93 \pm 3.09\text{g/m}^2$ and $+1.58 \pm 2.59\text{g/m}^2$ ($p=0.003$) for the allopurinol group and placebo group respectively. These changes are represented graphically in Figures 3.4.1 and 3.4.2. Conversely, when below median LVM and LVMI were analysed the change in LVM and LVMI becomes non-significant. For the below median baseline LVM sub-group, change in LVM was $-1.27 \pm 4.76\text{g}$ and $-0.16 \pm 5.10\text{g}$ ($p=0.559$) for the allopurinol group and placebo group respectively. Change in LVMI in the below median LVM group was $-0.71 \pm 2.52\text{g/m}^2$ and $+0.02 \pm 2.70\text{g/m}^2$ ($p=0.552$) for the allopurinol group and placebo group respectively. For the below median baseline LVMI sub-group, change in LVM was $-1.27 \pm 4.76\text{g}$ and $0.95 \pm 4.59\text{g}$ ($p=0.240$) for the

allopurinol group and placebo group respectively. Change in LVMI in the below median LVMI group was $-0.71 \pm 2.52\text{g/m}^2$ and $-0.50 \pm 3.31\text{g/m}^2$ ($p=0.299$) for the allopurinol group and placebo group respectively. This suggests the patients with the highest LVM at baseline have the most to gain from regression in LVM, which makes sense, as LVM can never be reduced to zero. As for the other parameters measured on CMR, sub-group analysis of above and below median LVM showed no new trends, as shown in Table 3.4.1 and 3.4.2.

| | Placebo (LVM above median) | Allopurinol (LVM above median) | P value | Placebo (LVM below median) | Allopurinol (LVM below median) | P value |
|---------------------------------|-------------------------------------|---|---------|-------------------------------------|---|---------|
| Change LVM (g) | 2.67±4.87 | -4.03±6.77 | 0.006 | -0.16±5.10 | -1.27±4.76 | 0.559 |
| Change LVMI (g/m ²) | 1.32±2.13 | -1.93±3.09 | 0.004 | 0.02± 2.70 | -0.71±2.52 | 0.552 |
| | | | | | | |
| Change in EF (mls) | -0.64±9.17 | 0.26±5.48 | 0.913 | 0.22±5.63 | 2.66±6.67 | 0.303 |
| Change in EDV (mls) | - 0.75±44.45 | 5.81±40.28 | 0.692 | 9.30±35.34 | 6.43±42.50 | 0.847 |
| Change in ESV (mls) | 3.09±30.63 | 1.78±15.00 | 0.889 | 1.83±10.64 | - 1.38±14.61 | 0.507 |
| Change in SV (mls) | - 3.83±21.28 | 3.99±27.90 | 0.418 | 7.47±27.32 | 7.80±30.85 | 0.977 |
| Change in CO (L/min) | -0.23±1.65 | 0.68±1.07 | 0.107 | 0.07±1.62 | 0.65±1.81 | 0.373 |

Table 3.4.1. Changes in measured parameters analysed as sub-groups of above and below median LVM

| | Placebo (LVMI above median) | Allopurinol (LVMI above median) | P value | Placebo (LVMI below median) | Allopurinol (LVMI below median) | P value |
|---------------------------------|--------------------------------------|--|---------|--------------------------------------|--|---------|
| Change LVM (g) | 1.42±5.63 | -4.03±6.77 | 0.025 | 0.95±4.59 | -1.27±4.76 | 0.240 |
| Change LVMI (g/m ²) | 1.58±2.59 | -1.93±3.09 | 0.003 | -0.50±3.31 | -0.71±2.52 | 0.299 |
| | | | | | | |
| Change in EF (mls) | -0.13±9.67 | 0.26±5.48 | 0.899 | 0.35±3.36 | 2.66±6.67 | 0.274 |
| Change in EDV (mls) | 1.44±41.48 | 5.81±40.28 | 0.778 | 8.16±38.48 | 6.43±42.50 | 0.062 |
| Change in ESV (mls) | 3.30±28.47 | 1.78±15.00 | 0.864 | 1.38±9.86 | - 1.38±14.61 | 0.110 |
| Change in SV (mls) | - 1.85±20.38 | 3.99±27.90 | 0.520 | 6.77±29.57 | 7.80±30.85 | 0.932 |
| Change in CO (L/min) | -0.18±1.65 | 0.68±1.07 | 0.117 | 0.06±1.63 | 0.65±1.81 | 0.383 |

Table 3.4.2. Changes in measured parameters analysed as sub-groups of above and below median LVMI

All measured parameters were also divided into sub-groups of above and below median LVM and LVMI, see Tables 3.4.3, 3.4.4., 3.4.5. and 3.4.6. This sub-group analysis revealed no new changes or trends in measured

parameters. Of note there was no significant changes renal function, BNP, markers of OS and BP. Furthermore, no significant differences or trends are seen in FMD and Alx in the sub-group analysis.

| | Placebo (LVM above median) | Allopurinol (LVM above median) | P value | Placebo (LVM below median) | Allopurinol (LVM below median) | P value |
|-----------------------------------|---|---|----------------|---|---|----------------|
| Haemoglobin (g/L) | -0.53±0.57 | -0.45±0.56 | 0.735 | -2.39±4.36 | -2.76±4.18 | 0.822 |
| Creatinine (µm/L) | 4.21±10.15 | -5.92±7.44 | 0.625 | -5.07±8.89 | -6.46±5.41 | 0.627 |
| eGFR (ml/min//1.73 ²) | -0.19±0.42 | -0.95±2.57 | 0.284 | 0.053±0.51 | -0.12±0.48 | 0.380 |
| Fasting glucose (mmol/L) | 2.38±5.37 | 0.03±3.79 | 0.205 | -0.67±1.50 | 0.53±4.00 | 0.288 |
| HBA1c (%) | 3.66±16.88 | 1.18±13.48 | 0.421 | 7.83±21.76 | 5.14±18.86 | 0.731 |
| Urine PCR (mg/mmol) | 6.93±14.90 | 0.77±6.37 | 0.181 | -1.27±9.80 | 10.23±29.13 | 0.161 |
| BNP (pg/ml) | 4.46±24.09 | 3.27±25.44 | 0.902 | 0.93±14.86 | -5.67±14.66 | 0.405 |
| Uric acid (6 months) (mmol/L) | 0.00±0.08 | -0.23±0.13 | <0.001 | -0.01±0.06 | -0.28±0.19 | <0.001 |
| Uric acid (9 months) (mmol/L) | 0.00±0.063 | -0.21±0.17 | <0.001 | 0.03±0.05 | -0.27±0.17 | <0.001 |
| HS TropT (ng/L) | -0.11±3.84 | 0.56±1.50 | 0.561 | 0.29±0.80 | -0.89±2.73 | 0.122 |
| Oxidised LDL (U/L) | -0.41±5.78 | 2.41±8.73 | 0.328 | -0.43±9.24 | -0.88±45 | 0.900 |

Table 3.4.3. Sub-group analysis of above and below median baseline LVM for change in blood parameters (Over 9 month study period)

| | Placebo (LVMI above median) | Allopurinol (LVMI above median) | P value | Placebo (LVMI below median) | Allopurinol (LVMI below median) | P value |
|----------------------------------|--|--|----------------|--|--|----------------|
| Haemoglobin (g/L) | -0.44±0.50 | -0.45±0.56 | 0.935 | -2.79±4.58 | -2.76±4.18 | 0.986 |
| Creatinine (µm/L) | -3.00±9.71 | -5.92±7.44 | 0.380 | -6.69±8.85 | -6.46±5.41 | 0.937 |
| eGFR (ml/min/1.73 ²) | -0.14±0.43 | -0.95±2.57 | 0.220 | 0.02±0.53 | -0.12±0.48 | 0.495 |
| Fasting glucose (mmol/L) | 1.71±5.18 | 0.03±3.79 | 0.338 | -0.32±1.87 | 0.53±4.00 | 0.492 |
| HBA1c (%) | 4.28±15.78 | 1.18±13.48 | 0.332 | 7.71±23.52 | 5.14±18.86 | 0.761 |
| Urine PCR (mg/mmol) | 5.00±15.09 | 0.77±6.37 | 0.355 | -0.15±9.62 | 10.23±29.13 | 0.234 |
| BNP (pg/ml) | 0.47±20.75 | 3.27±25.44 | 0.746 | 5.29±18.48 | -5.67±14.66 | 0.956 |
| Uric acid (6 months) (mmol/L) | -0.00±0.08 | -0.23±0.13 | <0.001 | 0.00±0.05 | -0.28±0.19 | <0.001 |
| Uric acid (9 months) (mmol/L) | -0.01±0.06 | -0.21±0.17 | <0.001 | -0.01±0.06 | -0.27±0.17 | <0.001 |
| HS TropT (ng/L) | -0.10±3.59 | 0.56±1.50 | 0.539 | 0.33±0.82 | -0.89±2.73 | 0.134 |
| Oxidised LDL (U/L) | -0.28±7.05 | 2.41±8.73 | 0.366 | -0.59±8.58 | -0.88±4.5 | 0.936 |

Table 3.4.4. Sub-group analysis of above and below median baseline LVMI

for change in blood parameters (Over 9 month study period)

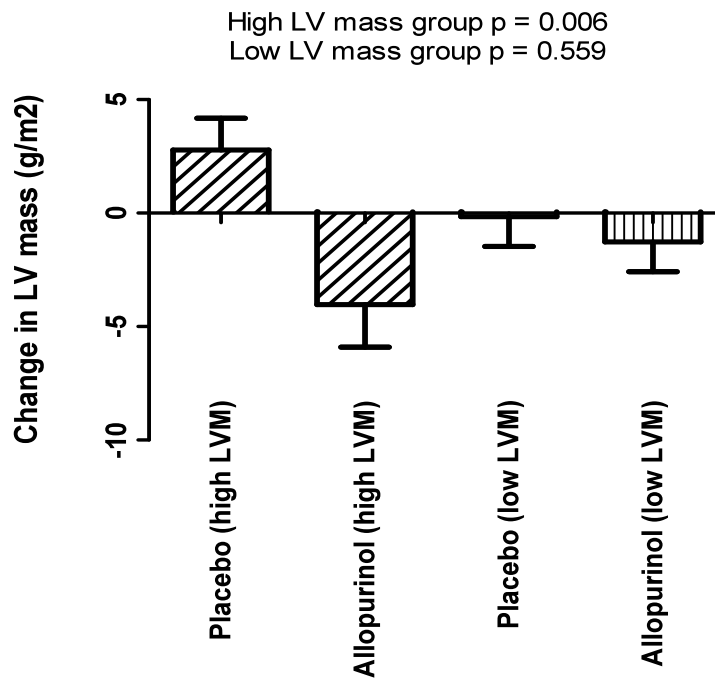
| | Placebo (LVM above median) | Allopurinol (LVM above median) | P value | Placebo (LVM below median) | Allopurinol (LVM below median) | P value |
|--|---|---|----------------|---|---|----------------|
| Office SBP (6 months) | 6.71±10.52 | 10.24±11.07 | 0.403 | 7.37±12.99 | 9.18±9.21 | 0.679 |
| Office SBP (9 months) | 5.50±18.07 | -8.31±6.26 | 0.600 | 3.00±10.30 | 9.18±9.21 | 0.253 |
| Office DBP (6 months) | 6.64±15.96 | -4.15±13.47 | 0.666 | 0.73±18.40 | -0.77±8.15 | 0.995 |
| Office DBP (9 months) | 8.21±18.73 | -4.38±14.04 | 0.556 | 5.27±37.01 | 4.54±13.67 | 0.376 |
| 24hr SBP (9 months) | -0.14±0.86 | -0.08±0.28 | 0.795 | 0.667±0.26 | 0.62±1.56 | 0.106 |
| | | | | | | |
| FMD hyperaemia (6 months) (%) | -0.47±3.84 | 1.40±3.16 | 0.182 | -0.72±1.96 | -1.65±4.95 | 0.508 |
| FMD hyperaemia (9 months) (%) | -0.26±4.65 | 1.24±3.84 | 0.373 | 1.14±4.31 | 0.76±9.68 | 0.894 |
| FMD GTN (6 months) (%) | 0.69±.55 | 0.62±3.96 | 0.964 | 2.09±6.95 | -0.85±8.19 | 0.312 |
| FMD GTN (9 months) (%) | 1.72±4.60 | 0.59±4.27 | 0.511 | 0.49±5.30 | 1.49±7.72 | 0.690 |
| | | | | | | |
| Alx (6 months) (%) | 1.02±11.11 | -2.33±10.13 | 0.297 | -3.40±.73 | 0.02±13.99 | 0.429 |
| Alx (9 months) (%) | 0.57±3.04 | -4.26±10.23 | 0.906 | 1.08±12.46 | 1.18±9.14 | 0.483 |

Table 3.4.5. Sub-group analysis of above and below median baseline LVM for change in parameters of endothelial function and blood pressure

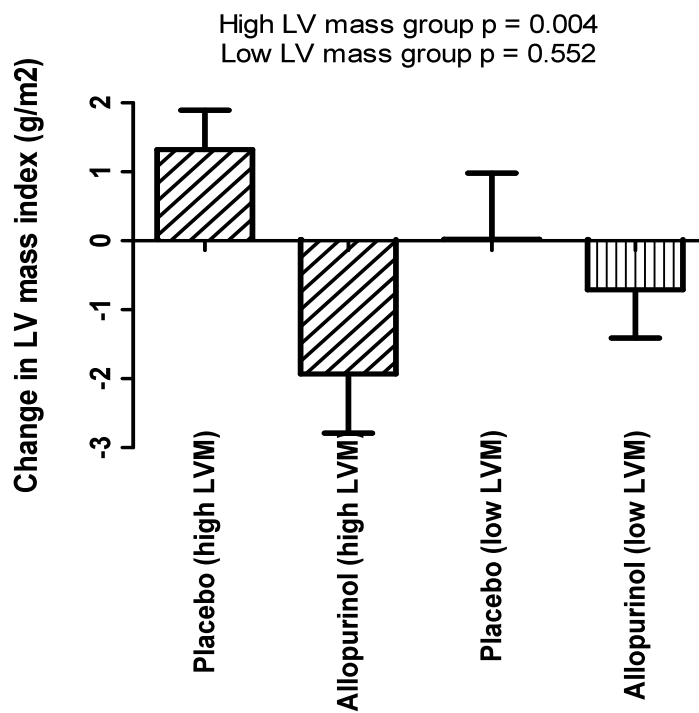
| | Placebo (LVMI above median) | Allopurinol (LVMI above median) | P value | Placebo (LVMI below median) | Allopurinol (LVMI below median) | P value |
|--|--|--|----------------|--|--|----------------|
| Office SBP (6 months) | 4.41±10.24 | 10.24±11.07 | 0.153 | 10.30±12.85 | 9.18±9.21 | 0.800 |
| Office SBP (9 months) | 4.31±16.87 | -8.31±6.26 | 0.427 | -4.08±11.20 | 9.18±9.21 | 0.448 |
| Office DBP (6 months) | 3.19±16.51 | -4.15±13.47 | 0.866 | -4.08±18.73 | -0.77±8.15 | 0.565 |
| Office DBP (9 months) | 14.06±35.74 | -4.38±14.04 | 0.367 | 2.38±15.00 | 4.54±13.67 | 0.705 |
| 24hr SBP (9 months) | -0.13±0.81 | -0.08±0.28 | 0.839 | -0.77±0.28 | 0.62±1.56 | 0.127 |
| | | | | | | |
| FMD hyperaemia (6 months) (%) | -0.56±3.56 | 1.40±3.16 | 0.132 | -0.64±2.15 | -1.65±4.95 | 0.505 |
| FMD hyperaemia (9 months) (%) | -0.03±4.57 | 1.24±3.84 | 0.431 | 1.07±4.42 | 0.76±9.68 | 0.917 |
| FMD GTN (6 months) (%) | 1.40±4.11 | 0.62±3.96 | 0.610 | 1.43±7.07 | -0.85±8.19 | 0.454 |
| FMD GTN (9 months) (%) | 1.93±4.54 | 0.59±4.27 | 0.421 | 0.05±5.37 | 1.49±7.72 | 0.585 |
| | | | | | | |
| Alx (6 months) (%) | -0.96±10.39 | -2.33±10.13 | 0.723 | -3.84±8.19 | 0.02±13.99 | 0.404 |
| Alx (9 months) (%) | 0.10±11.98 | -4.26±10.23 | 0.308 | -1.59±13.70 | 1.18±9.14 | 0.550 |

Table 3.4.6. Sub-group analysis of above and below median baseline LVM for change in parameters of endothelial function and blood pressure

(1)*



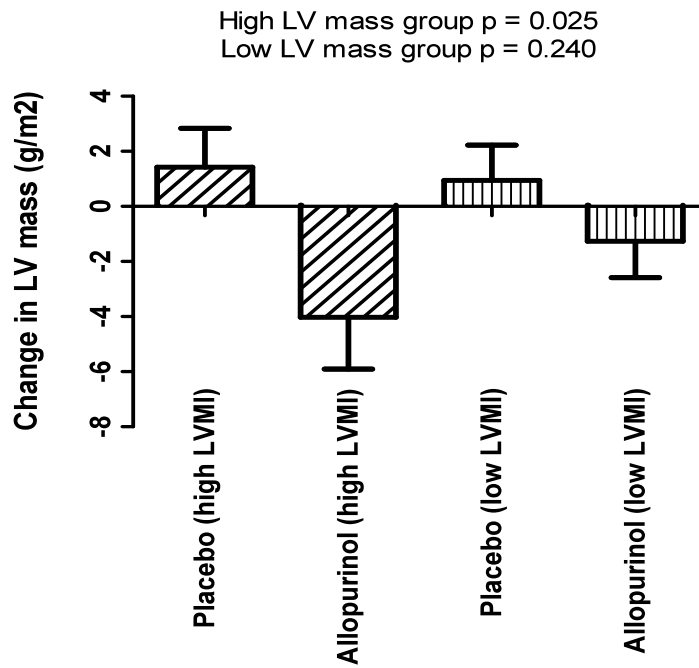
(2)*



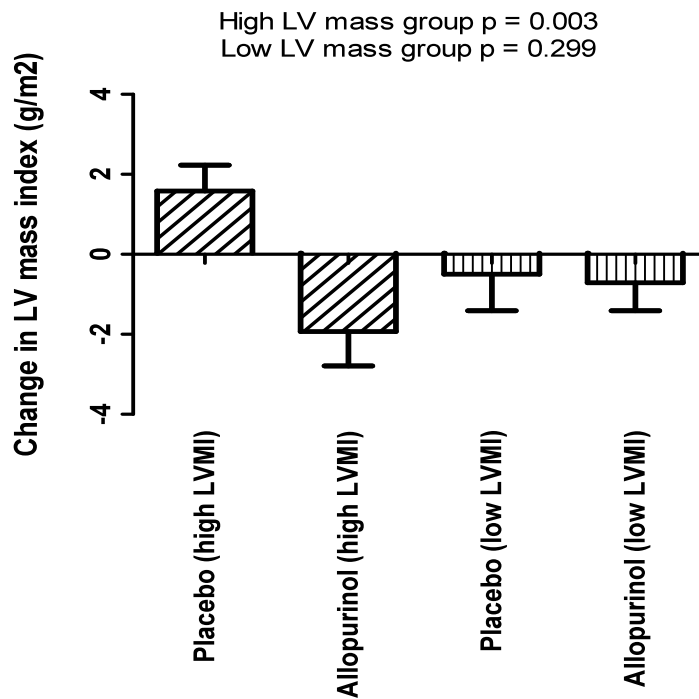
*Standard Deviation

Figure 3.4.1 Graphs of above and below median LVM changes in (1) LVM and (2) LVMI

(1)*



(2)*



*Standard Deviation

Figure 3.4.2. Graphs of above and below median LVMI changes in (1) LVM and (2) LVMI

5. Correlations

There was no correlation shown between change in blood parameters and change in LVM and LVMI. There was a trend seen with change in uric acid and change in LVM with a p value of 0.09. Although not reaching significance it does hint toward a trend that is probably explained by the fact that the group where the LVM fell was the allopurinol patients and hence there was a significant fall in uric acid. Baseline FMD correlated significantly with change in LVM and LVMI. Finally, a positive correlation was seen between change in LVM and LVMI and change in office systolic BP. This could be meaningful and leaves open the possibility that subtle changes in BP might be a factor in our results. Correlation results are shown in Table 3.5.1.

| | Change LVM | Change LVMI |
|-----------------------|--------------------------|--------------------------|
| Change in Uric Acid | r = 0.228 p = 0.09 | r = 0.223 p = 0.102 |
| Baseline Uric Acid | r = 0.201 p = 0.141 | r = 0.245 p = 0.072 |
| Change oxidised LDL | r = 0.068 p = 0.622 | r = 0.053 p = 0.698 |
| Baseline Oxidised LDL | r = -0.107 p = 0.437 | r = -0.035 p = 0.800 |
| Change BNP | r = 0.174 p = 0.203 | r = 0.198 p = 0.148 |
| Baseline BNP | r = -0.099 p = 0.470 | r = -0.144 p = 0.263 |
| Change HS Trop T | r = 0.248 p = 0.068 | r = 0.220 p = 0.107 |
| Baseline HS Trop T | r = 0.193 p = 0.158 | r = 0.094 p = 0.497 |
| Change PCR | r = -0.042 p = 0.759 | r = 0.013 p = 0.926 |
| Baseline PCR | r = 0.175 p = 0.201 | r = 0.125 p = 0.362 |
| Change Glucose | r = 0.026 p = 0.852 | r = 0.010 p = 0.945 |
| Baseline Glucose | r = 0.176 p = 0.198 | r = 0.066 p = 0.632 |
| Change Insulin | r = -0.172 p = 0.211 | r = -0.36 p = 0.794 |
| Baseline Insulin | r = 0.105 p = 0.444 | r = 0.077 p = 0.576 |
| Change eGFR | r = -0.067 p = 0.627 | r = -0.083 p = 0.545 |
| Baseline eGFR | r = -0.54 p = 0.694 | r = -0.062 p = 0.653 |
| Change FMD | r = 0.022 p = 0.870 | r = -0.039 p = 0.777 |
| Baseline FMD | r = 0.363 p = 0.007* | r = 0.300 p = 0.027* |
| Change Alx | r = -0.181 p = 0.187 | r = -0.143 p = 0.298 |
| Baseline Alx | r = -0.149 p = 0.284 | r = -0.164 p = 0.235 |
| Change Office SBP | r = -0.370 p = 0.005* | r = -0.342 p = 0.011* |
| Baseline Office SBP | r = 0.163 p = 0.234 | r = 0.169 p = 0.219 |
| Change 24 hour SBP | r = 0.039 p = 0.778 | r = 0.039 p = 0.780 |
| Baseline 24 hour SBP | r = 0.257 p = 0.539 | r = 0.285 p = 0.493 |

* = Significant correlation

Table 3.5.1. Correlation of LVM and LVMI with measured parameters.

6. **Important Drug Changes over Nine-Month Study Period**

Table 3.6.1 shows important changes made to study participants over the nine-month study period. As can be seen few patients had changes made to their medications. The most common change to medication was a titration of anti-diabetic drugs during the study.

| | Allopurinol Group | Placebo Group |
|----------------------------------|--------------------------|----------------------|
| New anti-hypertensive | 1 | 1 |
| Increase in anti-hypertensive | 1 | 0 |
| Titration of diabetic medication | 6 | 4 |

Table 3.6.1. Important drug changes over nine-month study period

CHAPTER 4**DISCUSSION**

The key finding from this study is that allopurinol reduces LVM in patients with T2DM and baseline LVH. All patients had an office BP at baseline below 150/90. There was no significant change in either office BP or 24 ambulatory BP over the nine-month study period. This is important because until recently the only way that has been shown to regress LVH was to decrease BP (79, 159, 161, 163). These findings are in keeping with two, recently published studies, showing similar findings. Kao *et al* (2011) was the first to show the effects of allopurinol on regression of LVM in patients with chronic kidney disease (236). These findings agree with Kao *et al* (2011), although it should be noted a higher dose of allopurinol was used in this study (600mg as compared with 300mg) over the same nine-month study period. Rekhraj *et al* (2013) also showed that allopurinol regresses LVM in patients with chronic stable angina (242). Their study was of similar design to this one, giving allopurinol 600mg over nine months. Again, this study showed no effects on BP. This is the third such study showing regression of LVM, which is compelling evidence the effects of allopurinol are very unlikely to be due to chance.

1. **Blood Pressure**

As BP has been previously strongly associated with development of LVH and the strategies for regression of LVH, the first issue to address is to whether allopurinol has any effects on BP, however subtle. We found no change in BP in the allopurinol group and other studies have also shown no significant effect of allopurinol on BP (211, 236). There has been one study that has shown allopurinol decreases BP in adolescents with newly diagnosed essential hypertension (237). This was a small cross-over study of 30 patients over a very short period of time (four weeks) and the fall in systolic BP was modest (-6mmHg). A similar finding was seen when allopurinol was given to 48 hyper-uricaemic patients and compared to normouricaemic patients. Allopurinol had small improvements in BP over three months in the hyper-uricaemic patients but there was no effect on BP in the normo-uricaemic patients (238). Conversely, all other studies using allopurinol in adults over long periods of time did not show any significant changes in BP, as was found in this study (211, 236). The difference here is that uric acid was not significantly raised in this or other studies quoted. There is an association between high uric acid and raised BP (240). It can be postulated that allopurinol may have small effects on BP in specific sub-sets of patients with high uric acid. Therefore it seems that allopurinol has minimal (if any) effect on BP over time in an unselected group of patients without high uric acid and high degrees of hypertension. It would seem therefore allopurinol is the first 'non anti-hypertensive drug' ever shown to regress LVH in man.

2. **Prognostic Benefits of Regressing LVH**

Regressing LVH improves CV morbidity and mortality irrespective of BP changes. Indeed, Schillaci *et al* (2004) claims in an editorial 'LVH stands out as the only available marker whose treatment induced regression has been unequivocally associated with a better prognosis, even after accounting for treatment induced BP reduction' (249). The largest trial to show this is the LIFE trial that studied hypertensive patients with signs of ECG LVH (77, 163). In LIFE, Devereux *et al* (2002) found that LVH regression *per se* (independent of BP) was overall associated with reduced all cause mortality (by 28%), CV mortality (by 38%), sudden cardiac death (by 19%), myocardial infarction (by 15%), new HF (by 36%), new AF (by 12%) and stroke (by 24%) (169, 171, 250).

A slightly unclear picture has arisen from the diabetic sub-study of the LIFE trial. Losartan was associated, over atenolol, with even fewer CV events and deaths in patients with diabetes than in those without diabetes (167). Losartan was also more effective than atenolol in reversing LVH in those with diabetes (167). Yet in an analysis of ECG-LVH changes *per se*, the presence of diabetes lessened the link between ECG-LVH changes and CV events. Indeed it appears from the LIFE study that reduction of BP does not regress ECG-LVH as much in patients with diabetes as it does in those without diabetes (168). This highlights the potential importance of finding novel ways to regress LVH in T2DM, like allopurinol. It is also worth noting that the change in LVM on CMR in this study is of similar magnitude to the changes

seen with allopurinol in a previous renal study which raises the possibility that, unlike BP reduction, allopurinol may regress LVH as well in patients with diabetes as in those without diabetes (236).

As the sub-group who conferred the highest change in LVM had the highest baseline LVM, this would suggest in the future studies are targeted toward patients with the highest baseline LVM as they may confer the most benefit. It is not surprising that LVM regression is greater in those the highest baseline LVM.

3. **Magnitude of Changes in Left Ventricular Mass**

The magnitude of the LVM changes we produced here appears modest. The difference between allopurinol and placebo was 3.2% for LVM and 3.3% for LVMI. When compared to other studies looking at regression of LVM the changes seen in this study are comparable. Simpson *et al* (2010), Kao *et al* (2011) and Rekhraj *et al* (2013) showed similar falls in LVM to this study with changes in LVM over time (one year and nine months respectively). In the echo sub-study of the LIFE study, the difference between the two active treatments was only 3% and yet when this was maintained over many years (4.8 years), the differential effect on CV events between the two treatments was a 14% reduction in CV events and a 25% reduction in strokes (165). These were the results in the main LIFE trial although the effects were even greater in the diabetic sub-study of LIFE where CV events were reduced by 24% and total mortality by 39% (167). The LIFE trial is in fact the best comparator for our trial because only the LIFE trial and our study eliminated the effect of BP on LVH regression. In any case, we only gave allopurinol for nine months and it would be expected that the small difference we saw in LVM would increase over the first two years of treatment as also occurred in the echo sub-study of the LIFE trial (165). The full LVH regressing effect of allopurinol may take two years to manifest itself.

The effect of regression of LVM is concentrated to the sub group of patients with highest baseline LVM. This is logical as the higher the LVM at baseline then the greater potential for it to regress.

4. Other Studies Regressing Left Ventricular Hypertrophy Using Allopurinol

There are now two direct comparisons to this trial, Kao *et al* (2011) and Rekhraj *et al* (2013) both of similar design. In both these studies allopurinol was given over the same time frame of nine months (236, 242). Kao *et al* (2011) gave allopurinol at a dose of 300mg over nine months to patients with chronic kidney disease stage 3 and LVH. The primary end-point was change in LVM in active and placebo arms. Their findings were similar to this trial with significant fall in LVMI (change in LVMI at nine months for the allopurinol group $-1.42 \pm 4.67\text{g/m}^2$ and for the placebo group $1.28 \pm 4.45\text{g/m}^2$). Rekhraj *et al* (2013) found very similar results in 66 patients with chronic stable angina. Allopurinol was given at a higher dose in this study, 600mg per day over nine months. LVM fell by $2.2 \pm 2.78\text{g/m}^2$ in the active group and rose by $0.53 \pm 2.5\text{g/m}^2$ in the placebo group. Although these trials were done on different cohorts of patients, allopurinol has now consistently regressed LVM in two other randomised control trials. The findings in this trial are consistent with three experimental studies in animal models that have also shown that allopurinol regresses LVH (225, 251, 252).

5. Reducing Cardiovascular Risk Further in Diabetes

Improving mortality and morbidity in diabetes is important. The incidence of diabetes is increasing and CV disease is a key reason for increased morbidity and mortality. Although it may be overstating it, death from CV disease has in general fallen over the last 35 years but there has not been a decline in CV deaths in T2DM (7). Therefore, it is vitally important to find new therapies that may reduce CV burden in diabetes, despite aggressive treatments for IHD. LVH is common in diabetes (in fact, diabetes is a risk factor for LVH in its own right) and we know that LVH confers significant CV risk (32). Hence novel ways of regressing LVH in diabetic patients is one potential way of addressing this issue such as allopurinol.

6. **Possible Mechanisms Why Allopurinol Reduces Left Ventricular Mass**

In this study our secondary end-points do not give any clues as to why allopurinol may regress LVH. There were no significant changes seen in FMD, A1x or tissue markers of OS (oxidised LDL) or BNP. There was also no change in other parameters measured on CMR. The most obvious reason for these findings could be relatively small numbers of patients recruited and short follow period of the trial which prevented our seeing small changes in oxidised LDL and BNP.

George *et al* (2006) is one of the best examples of the effects of allopurinol on FMD, especially the higher dose of 600mg (211). They showed that allopurinol 600mg improved endothelial function by 143% compared to placebo in patients with chronic heart failure. Allopurinol has improved endothelial function in various other patient groups including smokers, hypertension and diabetes (212, 215, 229). Allopurinol has been shown to reduce tissue OS and we know OS is a mediator of LVH (95). Therefore it could be the reduction in OS that mediated LVH regression as discussed above. However we found no change in oxidised LDL. ROS are only detectable in plasma for very short periods of time and this study is not unique in not changing plasma biomarkers of OS. This does not preclude an effect on OS as plasma biomarkers tend to change very little (if at all) after allopurinol whereas vascular tissue OS has been shown to change profoundly following allopurinol (211, 245). This disconnect between plasma OS

biomarkers and tissue OS was particularly obvious in George *et al* (2006) and Rajendra *et al* (2010). Overall current plasma OS biomarkers may be fairly insensitive in this situation with allopurinol. The reason for a lack of effect of allopurinol on endothelial function in this study might be that our T2DM patients were very well treated with statins, ACE inhibitors and ARB's which are each known to improve endothelial function. Indeed, this was evident in the baseline measures of vascular health in our patients. The baseline AIx was only 11% and therefore, it may have made it difficult to improve it further with allopurinol. This notion is in general supported by a previous study in a cohort of T2DM patients whose co-treatment was not as optimal as in our cohort. In that study, allopurinol was able to improve endothelial function in patients with T2DM but had no effect on endothelial function in healthy individuals who had much better endothelial function at baseline than the T2DM patients (212). Another reason why we were able to demonstrate an effect of allopurinol on LVH but not on endothelial function in this study may have been because we pre-selected those with baseline LVH whereas abnormal endothelial function at baseline was not a prerequisite in this study: this was obviously because our primary end-point was LVM but it may have made it easier to change LVM because it was elevated. This theory is further confirmed as when high and low LVM was analysed separately a bigger fall in LVM was observed.

Benzbromarone has shown improvements in fasting insulin in 82 patients with heart failure (253). Benzbromarone is a potent uricosuric drug that has been used previously in the treatment of gout but it has been withdrawn from the

market due to fears surrounding hepatotoxicity (254). Questions have been raised about how allopurinol affects fasting insulin and in this study had it no effects on fasting insulin. This may be due to small numbers of patients and short follow-up, but if allopurinol were to improve fasting insulin, like benzobromarone, this may be another mechanism whereby it might decrease OS.

The second possibility is that LVH might regress due to a reduction in LV after-load and some studies have found allopurinol reduces A1x although this was not seen here (231, 236). Allopurinol has also been shown to reduce BNP in chronic heart failure (232). With regards to BNP not falling in this study, baseline BNP was not particularly high therefore there was probably little scope to see a significant fall in BNP.

A third possible reason is that energy depletion may produce hypertrophy and in small studies allopurinol has improved energetics (255, 256). In hypertrophic cardiomyopathy, a genetic condition causing LVH, one hypothesis based on observations of mutant contractile protein function, together with mouse models and clinical studies, suggests that sarcomeric hypertrophic cardiomyopathy mutations lead to inefficient ATP utilisation (256). The suggestion that energy depletion underlies hypertrophic cardiomyopathy may have implications for therapy in hypertrophic cardiomyopathy. This could be extended to LVH due to other causes (e.g. hypertension and OS) and recently it has been shown intravenous allopurinol acutely improves the relative and absolute concentrations of myocardial high-

energy phosphates and ATP flux through creatinine kinase in heart failure (255).

Overall this study does not clarify the exact mechanism of allopurinol's effect on LVM in T2DM, although a number of mechanisms remain possible from previous studies. Irrespective of the mechanism, it is encouraging that allopurinol was able to regress LVH despite optimal medical treatment.

7. Allopurinol and its Effects on BNP

BNP can be raised in LVH and heart failure; we have not shown a change in BNP with allopurinol. This is in contrast to previous studies in heart failure and stroke patients (231). The change observed in this study on LVM was small and over a short period of time. It can be postulated that quite dramatic changes in LVM would need to be seen before a statistically significant change in BNP could be observed. The trial was powered to see a change in LVM, not BNP. Hence too few patients were probably recruited and followed up over a short period of change to see anything more than a trend in BNP improvement (placebo -1.49 ± 20.22 vs. allopurinol -3.16 ± 20.36 , $p = 0.754$). If allopurinol truly does change BNP, this suggests that allopurinol is decreasing after load probably via an effects on OS.

8. Effects of Allopurinol on other Cardiac MRI Parameters

Over the nine-month study period there were no other significant changes observed in other parameter measured on CMR, namely EF, EDV, ESV, SV and CO. We would not necessarily expect to see an improvement in EF, SV or CO as we have postulated allopurinol improves LVH by mechanisms that would not affect these parameters. However, allopurinol has improved ventricular volumes in other studies. Kao *et al* (2011) showed a trend towards an improvement in EDV (just not reaching significance $p = 0.084$) in their study of patients with chronic kidney disease. In mouse models allopurinol limited the increase in EDV after coronary artery ligation, and similar findings have been shown in rat and rabbit models with heart failure (257-259). In Rekhraj *et al* (2013) allopurinol significantly reduced ESV. These observations may be due to allopurinol improving mechanoenergetic uncoupling or it reducing LV afterload (216). In this study we did not see any change in EDV or ESV and this may have been due to the small numbers recruited or the short follow up period or due to the fact that LV afterload (as measured by A1x) did not fall in this study.

9. **Association of Uric Acid with Cardiovascular Disease**

High uric acid levels have been shown to be associated with increased risk of CV morbidity and mortality and hypertension. Verdeccia *et al* (2000) showed that in untreated subjects with essential hypertension, raised uric acid is a powerful risk marker for subsequent CV and all-cause mortality (260). It has been postulated that uric acid level itself is associated with an increased risk of having LVH and other target organ damage from hypertension (261, 262). In fact Viazzi *et al* (2005) showed each standard deviation increase in serum uric acid entailed a 75% higher risk of having LVH and a two-times greater risk of having carotid abnormalities (261). Also, uric acid is an anti-oxidant in it's own right (263), so therefore may play a role in the regression of LVH in this study. George *et al* (2006) specifically addressed this question (211). They showed that allopurinol improved FMD, whereas probenecid did not. Therefore it would seem that allopurinol improves endothelial function by reducing OS and not by reducing uric acid. In this study there was no correlation between baseline and change in uric acid levels and change in LVM or FMD, which supports this theory. Kao *et al* (2011) also found no correlation between baseline or change in urate with FMD or LVM (236).

10. Dose Response of Allopurinol

There appears to be an important dose response relationship with allopurinol. A lot of previous research using allopurinol had been with 300mg once daily (214). However, George *et al* (2006) showed that increasing the dose of allopurinol from 300mg to 600mg improved endothelial function by a further 52% such that allopurinol 600mg improved endothelial function by 143% compared to placebo (211). Previous studies using 300mg allopurinol may actually have used a suboptimal dose of allopurinol, hence our choice of dosing. Allopurinol was well tolerated in our study at this high dose. Allopurinol can be given up to 800mg or 900mg per day, according to the FDA and British National Formulary respectively.

11. Effects of Allopurinol on Hyperglycaemia

It is worth noting that there had been previous reports of the effect of allopurinol on glycaemia. At least one case report suggests that allopurinol can induce new T2DM whereas one large database suggests exactly the opposite (264). Our study is the largest there is to study the effect of allopurinol on glycaemic control in T2DM and we have shown fairly conclusively that allopurinol has no positive or negative effect on glycaemic control in T2DM. We also found that high dose allopurinol has no adverse effect on renal function in T2DM.

12. Side Effects and Tolerance of Allopurinol

Allopurinol was very well tolerated in this study at a high dose of 600mg. There were no effects on renal function and liver function tests over the nine-month study period. There was one true side effect, which was a rash. This is in keeping with other studies done within our department. It should be noted that all recruited patients did not have impaired renal function or heart failure and this might help explain the high tolerance rate.

13. Clinical Relevance

In this study we have shown that allopurinol regresses LVH in patients with T2DM and controlled BP. However, this study was too small to examine CV outcomes over time. There are a number of reasons why it can be hoped that allopurinol might improve prognosis if given to patients T2DM and LVH. As previously discussed, the best comparator is the LIFE study where regressing LVH was associated with improved CV outcomes. We know from the echo sub-study of LIFE that the difference between the two active treatments was only 3% compared with this study where the difference between allopurinol and placebo was 3.2% for LVM and 3.3% for LVMI. In LIFE there was a 14% reduction in CV events and a 25% reduction in strokes (165). Hence this study could be very clinically relevant if we extrapolate the LIFE improvement in CV outcomes with small reductions in LVM to our study. Furthermore, this study was conducted over a relatively short time period of nine months; one could hope for further reductions in LVM over years. We know allopurinol usually has beneficial effects on endothelial function, although we did not show that here. Struthers *et al* (2002) has shown in a retrospective cohort study of heart failure patients that long-term low dose allopurinol was associated with a significant worsening in mortality over those who never received allopurinol (228). Long-term high dose allopurinol was associated with a significantly better mortality than longstanding low dose allopurinol. MacDonald *et al* (2009) showed very similar findings in heart failure patients using record linkage (265). This may be due to the generally improved CV health through reduced OS, urate-lowering effects or even some unlooked for

regression of LVM. It raises the possibility that allopurinol may have future beneficial effects in patients with diabetes not only due to regression of LVH but also due to it improving endothelial function.

14. Limitations

The main limitation is the relatively small numbers of patients recruited. It is almost inevitable with so many demographic parameters that a few will by chance be different at baseline, although few differences were statistically significant. However, because of the relatively small sample size, we cannot exclude the possibility that some subtle demographic differences between the two groups may have contributed to our results.

The baseline LVMI in this study was not particularly high. This was due in part to the segmentation process, where the physicist actively excluded 'partial volume' (defined as <50% full thickness myocardium) areas at the extreme basal end of the ventricle. The inclusion or exclusion of a single slice in this region is known to alter the outcome value for the LVMI by typically 10%. However the emphasis at all stages of this work was to ensure optimised repeatability in order to maximise the potential sensitivity of the primary endpoint (change in LVMI).

The effect of allopurinol on LVM is modest but is similar in percentage terms to the differential effect in the echo sub-study of the LIFE trial which clearly delivered major benefits, especially in T2DM patients (165). Furthermore, we only gave treatment for nine months and the echo sub-study of LIFE suggests that full LVH regression takes longer, usually two years (165).

15. Future Research

a. Cardiovascular Outcomes

A large, multi-centre trial including follow up of CV outcomes over a longer time period would be the logical next step. Allopurinol has been shown to regress LVH in chronic kidney disease patients and chronic stable angina patients, therefore a larger trial could encompass a range of pathologies. We have shown in this study that allopurinol confers most benefit in those with highest baseline LVM, therefore selecting patients with highest LVM may help to target those who will gain most benefit.

b. Myocardial Fibrosis

One effect of allopurinol as yet untested is effects on fibrosis of the myocardium associated with LVH. This is because OS might stimulate myocardial fibrosis. A future study in similar design incorporating gadolinium enhancement on CMR would be one way of assessing this further.

c. Left Atrial Size

The effects of LVH include diastolic dysfunction, or heart failure with normal ejection fraction. Here LVH often leads to LA dilatation and future studies could involve measurements of LA volume. An interesting hypothesis is that if

LVH is regressed and LA size reduced then this may have benefits in preventing AF developing.

16. Prizes, Publications and Presentations

a. Prizes

Winner of 'Best Poster' for all poster submissions, American College of Cardiology Annual Scientific Sessions. San Francisco, 2013. Abstract 'Allopurinol Reduces Left Ventricular Mass in Patients with Type II Diabetes and Left Ventricular Hypertrophy'.

Tayside Institute of Clinical Research (TICR) college symposium prize-winner 2012. Awarded £500 for significant achievement in the field of Cardiovascular Research for study 'Allopurinol Reduces Left Ventricular Mass in Patients with Type II Diabetes and Left Ventricular Hypertrophy'.

b. Publications

Szwejkowski BR, Gandy SJ, Rekhraj S, Houston G, Lang CC, Morris AD, George J, Struthers AD. Allopurinol Reduces Left Ventricular Mass in Patients with Type 2 Diabetes and Left Ventricular Hypertrophy. Journal of American College of Cardiology. 2013 Dec 17;62(24):2284-93.

c. Poster Presentations

European Cardiac Society Annual Congress

2013 **Szwejkowski BR**, Gandy SJ, Rekhraj S, Houston G, Lang CC, George J, Struthers AD. Allopurinol Regresses Left Ventricular Hypertrophy Especially those with Highest Left Ventricular Mass.

British Cardiovascular Society

2013 **Szwejkowski BR**, Gandy SJ, Rekhraj S, Houston G, Lang CC, George J, Struthers AD. Allopurinol Reduces Left Ventricular Mass in Patients with Type 2 Diabetes and Left Ventricular Hypertrophy.

American College of Cardiology

2013 **Szwejkowski BR**, Gandy SJ, Rekhraj S, Houston G, Lang CC, George J, Struthers AD. Allopurinol Reduces Left Ventricular Mass in Patients with Type 2 Diabetes and Left Ventricular Hypertrophy.

Scottish Society of Physicians

2012 **Szwejkowski BR**, Gandy SJ, Rekhraj S, Houston G, Lang CC, George J, Struthers AD. Allopurinol Regresses Left Ventricular Hypertrophy in Patients with Diabetes and Angina.

17. **References**

1. World Health Organisation DoNDS. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications 1999.
2. Diabetes: State of the Nations 2006: Diabetes UK 2006.
3. Gonzalez ELM, Johansson S, Wallander M-A, Rodriguez LAG. Trends in the prevalence and incidence of diabetes in the UK: 1996-2005. *J Epidemiol Community Health*. 2009 April 1, 2009;63(4):332-6.
4. Geiss LS, Pan L, Cadwell B, Gregg EW, Benjamin SM, Engelgau MM. Changes in incidence of diabetes in U.S. adults, 1997-2003. *Am J Prev Med*. 2006 May;30(5):371-7.
5. SIGN 55: Management of Diabetes 2001.
6. Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes*. 1999 May 1, 1999;48(5):937-42.
7. Jaffar Ali R, Assad M. Current concepts of cardiovascular diseases in diabetes mellitus. *International journal of cardiology*. 2003;89(2):123-34.
8. Nesto RW. Correlation between cardiovascular disease and diabetes mellitus: current concepts. *Am J Med*. 2004 Mar 8;116 Suppl 5A(116):11S-22S.
9. Kannel WB. Lipids, diabetes, and coronary heart disease: insights from the Framingham Study. *Am Heart J*. 1985 Nov;110(5):1100-7.
10. Milicevic Z, Raz I, Beattie SD, Campaigne BN, Sarwat S, Gromniak E, et al. Natural History of Cardiovascular Disease in Patients With Diabetes: Role of hyperglycemia. *Diabetes Care*. 2008 February 1, 2008;31(Supplement_2):S155-60.
11. Goraya TY, Leibson CL, Palumbo PJ, Weston SA, Killian JM, Pfeifer EA, et al. Coronary atherosclerosis in diabetes mellitus: a population-based autopsy study. *J Am Coll Cardiol*. 2002 Sep 4;40(5):946-53.
12. Frey N, Olson EN. CARDIAC HYPERTROPHY: The Good, the Bad, and the Ugly. *Annual Review of Physiology*. 2003;65(1):45-79.
13. Rodrigues SL, D'Angelo L, Pereira AC, Krieger JE, Mill JG. Revision of the Sokolow-Lyon-Rappaport and Cornell voltage criteria for left ventricular hypertrophy. *Arq Bras Cardiol*. 2008;90(1):46-53.
14. Pewsner D, Juni P, Egger M, Battaglia M, Sundstrom J, Bachmann LM. Accuracy of electrocardiography in diagnosis of left ventricular hypertrophy in arterial hypertension: systematic review. *Bmj*. 2007;335(7622):711. Epub 2007 Aug 28.
15. Dawson A, Rana BS, Pringle SD, Donnelly LA, Morris AD, Struthers AD. How much echo left ventricular hypertrophy would be missed in diabetics by applying the Losartan Intervention For Endpoint Reduction electrocardiogram criteria to select patients for angiotensin receptor blockade? *J Hypertens*. 2004;22(7):1403-8.
16. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for Chamber Quantification: A Report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, Developed in Conjunction with the European Association of Echocardiography, a Branch of the European Society of Cardiology. *Journal of the American Society of Echocardiography*. 2005;18(12):1440-63.
17. Devlin AM, Moore NR, Ostman-Smith I. A comparison of MRI and echocardiography in hypertrophic cardiomyopathy. *Br J Radiol*. 1999;72(855):258-64.

18. Katz J, Milliken MC, Stray-Gundersen J, Buja LM, Parkey RW, Mitchell JH, et al. Estimation of human myocardial mass with MR imaging. *Radiology*. 1988;169(2):495-8.
19. Allison JD, Flickinger FW, Wright JC, Falls DG, 3rd, Prisant LM, VonDohlen TW, et al. Measurement of left ventricular mass in hypertrophic cardiomyopathy using MRI: comparison with echocardiography. *Magn Reson Imaging*. 1993;11(3):329-34.
20. Kuhl HP, Bucker A, Franke A, Maul S, Nolte-Ernsting C, Reineke T, et al. Transesophageal 3-dimensional echocardiography: in vivo determination of left ventricular mass in comparison with magnetic resonance imaging. *J Am Soc Echocardiogr*. 2000;13(3):205-15.
21. Simpson HJ, Gandy SJ, Houston JG, Rajendra NS, Davies JJ, Struthers AD. Left ventricular hypertrophy: reduction of blood pressure already in the normal range further regresses left ventricular mass. *Heart*. 2009;96(2):148-52.
22. Stewart GA, Foster J, Cowan M, Rooney E, McDonagh T, Dargie HJ, et al. Echocardiography overestimates left ventricular mass in hemodialysis patients relative to magnetic resonance imaging. *Kidney Int*. 1999;56(6):2248-53.
23. Clay S, Alfakih K, Radjenovic A, Jones T, Ridgway JP, Sivananthan MU. Normal range of human left ventricular volumes and mass using steady state free precession MRI in the radial long axis orientation. *Magma*. 2006;19(1):41-5. Epub 2006 Feb 14.
24. Malayeri AA, Johnson WC, Macedo R, Bathon J, Lima JA, Bluemke DA. Cardiac cine MRI: Quantification of the relationship between fast gradient echo and steady-state free precession for determination of myocardial mass and volumes. *J Magn Reson Imaging*. 2008;28(1):60-6.
25. Alfakih K, Plein S, Thiele H, Jones T, Ridgway JP, Sivananthan MU. Normal human left and right ventricular dimensions for MRI as assessed by turbo gradient echo and steady-state free precession imaging sequences. *J Magn Reson Imaging*. 2003;17(3):323-9.
26. Natori S, Lai S, Finn JP, Gomes AS, Hundley WG, Jerosch-Herold M, et al. Cardiovascular function in multi-ethnic study of atherosclerosis: normal values by age, sex, and ethnicity. *AJR Am J Roentgenol*. 2006;186(6 Suppl 2):S357-65.
27. Cain PA, Ahl R, Hedstrom E, Ugander M, Allansdotter-Johnsson A, Friberg P, et al. Age and gender specific normal values of left ventricular mass, volume and function for gradient echo magnetic resonance imaging: a cross sectional study. *BMC Med Imaging*. 2009;9:2.
28. Hudsmith LE, Petersen SE, Tyler DJ, Francis JM, Cheng AS, Clarke K, et al. Determination of cardiac volumes and mass with FLASH and SSFP cine sequences at 1.5 vs. 3 Tesla: a validation study. *J Magn Reson Imaging*. 2006;24(2):312-8.
29. Levy D, Anderson KM, Savage DD, Kannel WB, Christiansen JC, Castelli WP. Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors. The Framingham Heart Study. *Ann Intern Med*. 1988;108(1):7-13.
30. Ang DS, Pringle SD, Struthers AD. The cardiovascular risk factor, left ventricular hypertrophy, is highly prevalent in stable, treated angina pectoris. *Am J Hypertens*. 2007;20(10):1029-35.
31. Wright GA, Ang DS, Stonebridge PA, Belch JJ, Struthers AD. Left ventricular hypertrophy is present in one-half of newly-diagnosed peripheral arterial disease patients. *J Hypertens*. 2007;25(2):463-9.
32. Dawson A, Morris A, Struthers A. The epidemiology of left ventricular hypertrophy in type 2 diabetes mellitus. *Diabetologia*. 2005;48(10):1971-9.

33. Hara-Nakamura N, Kohara K, Sumimoto T, Lin M, Hiwada K. Glucose intolerance exaggerates left ventricular hypertrophy and dysfunction in essential hypertension. *Am J Hypertens*. 1994;7(12):1110-4.
34. Galderisi M, Anderson KM, Wilson PWF, Levy D. Echocardiographic evidence for the existence of a distinct diabetic cardiomyopathy (The Framingham Heart Study). *The American Journal of Cardiology*. 1991;68(1):85-9.
35. Struthers AD, Morris AD. Screening for and treating left-ventricular abnormalities in diabetes mellitus: a new way of reducing cardiac deaths. *The Lancet*. 2002;359(9315):1430-2.
36. Barrios V, Escobar C, Calderon A, Echarri R, Barrios S, Navarro-Cid J. Electrocardiographic left ventricular hypertrophy regression induced by an angiotensin receptor blocker-based regimen in hypertensive patients with diabetes: data from the SARA study. *J Renin Angiotensin Aldosterone Syst*. 2009;10(3):168-73. Epub 2009 Aug 3.
37. Levy D, Garrison R, Savage D, Kannel W, Castelli W. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med*. 1990 May 31, 1990;322(22):1561-6.
38. Vakili BA, Okin PM, Devereux RB. Prognostic implications of left ventricular hypertrophy. *American Heart Journal*. 2001;141(3):334-41.
39. Liao Y, Cooper RS, McGee DL, Mensah GA, Ghali JK. The relative effects of left ventricular hypertrophy, coronary artery disease, and ventricular dysfunction on survival among black adults. *Jama*. 1995;273(20):1592-7.
40. Ferdinand KC, Serrano CC, Ferdinand DP. Contemporary treatment of heart failure: is there adequate evidence to support a unique strategy for African-Americans? *Con position. Curr Hypertens Rep*. 2002;4(4):311-8.
41. WRITING GROUP MEMBERS, Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, et al. Heart Disease and Stroke Statistics--2009 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2009 January 27, 2009;119(3):e21-181.
42. Gardin JM, Wagenknecht LE, Anton-Culver H, Flack J, Gidding S, Kurosaki T, et al. Relationship of Cardiovascular Risk Factors to Echocardiographic Left Ventricular Mass in Healthy Young Black and White Adult Men and Women : The CARDIA Study. *Circulation*. 1995 August 1, 1995;92(3):380-7.
43. Gardin JM, Brunner D, Schreiner PJ, Xie X, Reid CL, Ruth K, et al. Demographics and correlates of five-year change in echocardiographic left ventricular mass in young black and white adult men and women: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Journal of the American College of Cardiology*. 2002;40(3):529-35.
44. Dunn FG, McLenachan J, Isles CG, Brown I, Dargie HJ, Lever AF, et al. Left ventricular hypertrophy and mortality in hypertension: an analysis of data from the Glasgow Blood Pressure Clinic. *Journal of Hypertension*. 1990;8(8):775-82.
45. Andren B, Lind L, Hedenstierna G, Lithell H. Impaired systolic and diastolic function and ventricular arrhythmia are common in normotensive healthy elderly men with left ventricular hypertrophy. *Coron Artery Dis*. 1999;10(2):111-7.
46. Opherk D, Mall G, Zebe H, Schwarz F, Weihe E, Manthey J, et al. Reduction of coronary reserve: a mechanism for angina pectoris in patients with arterial hypertension and normal coronary arteries. *Circulation*. 1984;69(1):1-7.
47. Hamasaki S, Al Suwaidi J, Higano ST, Miyauchi K, Holmes DR, Jr., Lerman A. Attenuated coronary flow reserve and vascular remodeling in patients with

- hypertension and left ventricular hypertrophy. *J Am Coll Cardiol*. 2000;35(6):1654-60.
48. Gimelli A, Schneider-Eicke J, Neglia D, Sambuceti G, Giorgetti A, Bigalli G, et al. Homogeneously reduced versus regionally impaired myocardial blood flow in hypertensive patients: two different patterns of myocardial perfusion associated with degree of hypertrophy. *J Am Coll Cardiol*. 1998;31(2):366-73.
 49. Vogt M, Motz W, Strauer BE. Coronary haemodynamics in hypertensive heart disease. *Eur Heart J*. 1992;13(Suppl D):44-9.
 50. Mizuno R, Fujimoto S, Saito Y, Nakamura S. Exercise-induced delayed onset of left ventricular early relaxation in association with coronary microcirculatory dysfunction in patients with diabetes mellitus. *J Card Fail*. 2009;16(3):211-7.
 51. Messerli FH, Ventura HO, Elizardi DJ, Dunn FG, Frohlich ED. Hypertension and sudden death. Increased ventricular ectopic activity in left ventricular hypertrophy. *Am J Med*. 1984;77(1):18-22.
 52. McLenachan JM, Henderson E, Morris KI, Dargie HJ. Ventricular arrhythmias in patients with hypertensive left ventricular hypertrophy. *N Engl J Med*. 1987;317(13):787-92.
 53. Siegel D, Cheitlin MD, Black DM, Seeley D, Hearst N, Hulley SB. Risk of ventricular arrhythmias in hypertensive men with left ventricular hypertrophy. *Am J Cardiol*. 1990;65(11):742-7.
 54. Ghali JK, Kadakia S, Cooper RS, Liao YL. Impact of left ventricular hypertrophy on ventricular arrhythmias in the absence of coronary artery disease. *J Am Coll Cardiol*. 1991;17(6):1277-82.
 55. Vester EG, Kuhls S, Ochiulet-Vester J, Vogt M, Strauer BE. Electrophysiological and therapeutic implications of cardiac arrhythmias in hypertension. *Eur Heart J*. 1992;13(Suppl D):70-81.
 56. Cosin Aguilar J, Hernandez Martinez A, Andres Conejos F. Mechanisms of ventricular arrhythmias in the presence of pathological hypertrophy. *Eur Heart J*. 1993;14(Suppl J):65-70.
 57. Chinali M, de Simone G, Liu JE, Bella JN, Oberman A, Hopkins PN, et al. Left atrial systolic force and cardiac markers of preclinical disease in hypertensive patients: the Hypertension Genetic Epidemiology Network (HyperGEN) Study. *Am J Hypertens*. 2005;18(7):899-905.
 58. Chinali M, de Simone G, Wachtell K, Gerds E, Gardin JM, Boman K, et al. Left atrial systolic force in hypertensive patients with left ventricular hypertrophy: the LIFE study. *J Hypertens*. 2008;26(7):1472-6.
 59. Cuspidi C, Meani S, Fusi V, Valerio C, Catini E, Sala C, et al. Prevalence and correlates of left atrial enlargement in essential hypertension: role of ventricular geometry and the metabolic syndrome: the Evaluation of Target Organ Damage in Hypertension study. *J Hypertens*. 2005;23(4):875-82.
 60. Gerds E, Oikarinen L, Palmieri V, Otterstad JE, Wachtell K, Boman K, et al. Correlates of left atrial size in hypertensive patients with left ventricular hypertrophy: the Losartan Intervention For Endpoint Reduction in Hypertension (LIFE) Study. *Hypertension*. 2002;39(3):739-43.
 61. Stritzke J, Markus MRP, Duderstadt S, Lieb W, Luchner A, Döring A, et al. The Aging Process of the Heart: Obesity Is the Main Risk Factor for Left Atrial Enlargement During Aging: The MONICA/KORA (Monitoring of Trends and Determinations in Cardiovascular Disease/Cooperative Research in the Region of Augsburg) Study. *Journal of the American College of Cardiology*. 2009;54(21):1982-9.

62. Gerds E, Wachtell K, Omvik P, Otterstad JE, Oikarinen L, Boman K, et al. Left atrial size and risk of major cardiovascular events during antihypertensive treatment: losartan intervention for endpoint reduction in hypertension trial. *Hypertension*. 2007;49(2):311-6. Epub 2006 Dec 18.
63. Benjamin EJ, D'Agostino RB, Belanger AJ, Wolf PA, Levy D. Left atrial size and the risk of stroke and death. The Framingham Heart Study. *Circulation*. 1995;92(4):835-41.
64. Bayes-Genis A, Vazquez R, Puig T, Fernandez-Palomeque C, Fabregat J, Bardaji A, et al. Left atrial enlargement and NT-proBNP as predictors of sudden cardiac death in patients with heart failure. *Eur J Heart Fail*. 2007;9(8):802-7. Epub 2007 Jun 13.
65. Rutter MK, Parise H, Benjamin EJ, Levy D, Larson MG, Meigs JB, et al. Impact of Glucose Intolerance and Insulin Resistance on Cardiac Structure and Function: Sex-Related Differences in the Framingham Heart Study. *Circulation*. 2003 January 28, 2003;107(3):448-54.
66. Lind L, Berne C, Andren B, Lithell H. Relationship between diastolic hypertension and myocardial morphology and function in elderly males with diabetes mellitus. *Diabetologia*. 1996;39(12):1603-6.
67. Sugishita K, Shiono E, Sugiyama T, Ashida T. Diabetes influences the cardiac symptoms related to atrial fibrillation. *Circ J*. 2003;67(10):835-8.
68. Gage BF, van Walraven C, Pearce L, Hart RG, Koudstaal PJ, Boode BSP, et al. Selecting Patients With Atrial Fibrillation for Anticoagulation: Stroke Risk Stratification in Patients Taking Aspirin. *Circulation*. 2004 October 19, 2004;110(16):2287-92.
69. Gage BF, Waterman AD, Shannon W, Boechler M, Rich MW, Radford MJ. Validation of Clinical Classification Schemes for Predicting Stroke: Results From the National Registry of Atrial Fibrillation. *JAMA*. 2001 June 13, 2001;285(22):2864-70.
70. Wilhelmsen L, Rosengren A, Lappas G. Hospitalizations for atrial fibrillation in the general male population: morbidity and risk factors. *J Intern Med*. 2001;250(5):382-9.
71. Movahed M, Hashemzadeh M, Jamal M. Diabetes mellitus is a strong, independent risk for atrial fibrillation and flutter in addition to other cardiovascular disease. *Int J Cardiol*. 2005;105:315 - 21.
72. Nichols GA, Reinier K, Chugh SS. Independent contribution of diabetes to increased prevalence and incidence of atrial fibrillation. *Diabetes Care*. 2009;32(10):1851-6.
73. Aksnes T, Schmieder R, Kjeldsen S, Ghani S, Hua T, Julius S. Impact of New-Onset Diabetes Mellitus on Development of Atrial Fibrillation and Heart Failure in High-Risk Hypertension (from the VALUE Trial). *Am J Cardiol*. 2008;101:634 - 8.
74. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of Atrial Fibrillation on the Risk of Death : The Framingham Heart Study. *Circulation*. 1998 September 8, 1998;98(10):946-52.
75. Ostgren CJ, Merlo J, Rastam L, Lindblad U. Atrial fibrillation and its association with type 2 diabetes and hypertension in a Swedish community. *Diabetes Obes Metab*. 2004;6(5):367-74.
76. Psaty BM, Manolio TA, Kuller LH, Kronmal RA, Cushman M, Fried LP, et al. Incidence of and risk factors for atrial fibrillation in older adults. *Circulation*. 1997;96(7):2455-61.
77. Devereux RB, Palmieri V, Liu JE, Wachtell K, Bella JN, Boman K, et al. Progressive hypertrophy regression with sustained pressure reduction in hypertension:

- the Losartan Intervention For Endpoint Reduction study. *J Hypertens.* 2002;20(7):1445-50.
78. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *The Lancet.* 2000;355(9200):253-9.
 79. Klingbeil AU, Schneider M, Martus P, Messerli FH, Schmieder RE. A meta-analysis of the effects of treatment on left ventricular mass in essential hypertension. *Am J Med.* 2003;115(1):41-6.
 80. Lorell BH, Carabello BA. Left Ventricular Hypertrophy : Pathogenesis, Detection, and Prognosis. *Circulation.* 2000 July 25, 2000;102(4):470-9.
 81. Kannel WB. Left ventricular hypertrophy as a risk factor in arterial hypertension. *Eur Heart J.* 1992;13(Suppl D):82-8.
 82. Lorber R, Gidding SS, Daviglus ML, Colangelo LA, Liu K, Gardin JM. Influence of systolic blood pressure and body mass index on left ventricular structure in healthy African-American and white young adults: the CARDIA study. *J Am Coll Cardiol.* 2003;41(6):955-60.
 83. Verdecchia P, Schillaci G, Boldrini F, Guerrieri M, Gatteschi C, Benemio G, et al. Risk stratification of left ventricular hypertrophy in systemic hypertension using noninvasive ambulatory blood pressure monitoring. *Am J Cardiol.* 1990;66(5):583-90.
 84. Cicconetti P, Morelli S, Ottaviani L, Chiarotti F, De Serra C, De Marzio P, et al. Blunted nocturnal fall in blood pressure and left ventricular mass in elderly individuals with recently diagnosed isolated systolic hypertension. *Am J Hypertens.* 2003;16(11 Pt 1):900-5.
 85. Cuspidi C, Macca G, Sampieri L, Fusi V, Severgnini B, Michev I, et al. Target organ damage and non-dipping pattern defined by two sessions of ambulatory blood pressure monitoring in recently diagnosed essential hypertensive patients. *J Hypertens.* 2001;19(9):1539-45.
 86. Chen CH, Ting CT, Lin SJ, Hsu TL, Ho SJ, Chou P, et al. Which arterial and cardiac parameters best predict left ventricular mass? *Circulation.* 1998;98(5):422-8.
 87. LEVY D, ANDERSON KM, SAVAGE DD, KANNEL WB, CHRISTIANSEN JC, CASTELLI WP. Echocardiographically Detected Left Ventricular Hypertrophy: Prevalence and Risk Factors: The Framingham Heart Study. *Ann Intern Med.* 1988 January 1, 1988;108(1):7-13.
 88. Karason K, Sjostrom L, Wallentin I, Peltonen M. Impact of blood pressure and insulin on the relationship between body fat and left ventricular structure. *Eur Heart J.* 2003;24(16):1500-5.
 89. Teuscher A, Egger M, Herman JB. Diabetes and hypertension. Blood pressure in clinical diabetic patients and a control population. *Arch Intern Med.* 1989;149(9):1942-5.
 90. Timmis AD. Diabetic heart disease: clinical considerations. *Heart.* 2001;85(4):463-9.
 91. Sawyer DB, Siwik DA, Xiao L, Pimentel DR, Singh K, Colucci WS. Role of Oxidative Stress in Myocardial Hypertrophy and Failure. *Journal of Molecular and Cellular Cardiology.* 2002;34(4):379-88.
 92. Seddon M, Looi YH, Shah AM. Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. *Heart.* 2007 August 1, 2007;93(8):903-7.
 93. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, et al. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet.* 1995;11(4):376-81.

94. Assem M, Teyssier JR, Benderitter M, Terrand J, Laubriet A, Javouhey A, et al. Pattern of superoxide dismutase enzymatic activity and RNA changes in rat heart ventricles after myocardial infarction. *Am J Pathol.* 1997;151(2):549-55.
95. Takimoto E, Kass DA. Role of Oxidative Stress in Cardiac Hypertrophy and Remodeling. *Hypertension.* 2007 February 1, 2007;49(2):241-8.
96. Siwik DA, Tzortzis JD, Pimental DR, Chang DL, Pagano PJ, Singh K, et al. Inhibition of copper-zinc superoxide dismutase induces cell growth, hypertrophic phenotype, and apoptosis in neonatal rat cardiac myocytes in vitro. *Circ Res.* 1999;85(2):147-53.
97. Kwon SH, Pimentel DR, Remondino A, Sawyer DB, Colucci WS. H₂O₂ regulates cardiac myocyte phenotype via concentration-dependent activation of distinct kinase pathways. *J Mol Cell Cardiol.* 2003;35(6):615-21.
98. Pimentel DR, Amin JK, Xiao L, Miller T, Viereck J, Oliver-Krasinski J, et al. Reactive oxygen species mediate amplitude-dependent hypertrophic and apoptotic responses to mechanical stretch in cardiac myocytes. *Circ Res.* 2001;89(5):453-60.
99. Cave A, Grieve D, Johar S, Zhang M, Shah AM. NADPH oxidase-derived reactive oxygen species in cardiac pathophysiology. *Philos Trans R Soc Lond B Biol Sci.* 2005 Dec 29;360(1464):2327-34.
100. Sirker A, Zhang M, Murdoch C, Shah AM. Involvement of NADPH Oxidases in Cardiac Remodelling and Heart Failure.
101. Murdoch CE, Zhang M, Cave AC, Shah AM. NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure. *Cardiovasc Res.* 2006 July 15, 2006;71(2):208-15.
102. Finkel T. Signal transduction by reactive oxygen species in non-phagocytic cells. *J Leukoc Biol.* 1999;65(3):337-40.
103. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res.* 1058;107(9):1058-70.
104. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* 1980;288(5789):373-6.
105. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A.* 1987;84(24):9265-9.
106. Stevens A LJ, editor. *Human Histology.* 3rd ed: Elsevier Mosby; 2008.
107. Vallance P. Endothelial regulation of vascular tone. *Postgrad Med J.* 1992;68(803):697-701.
108. Ignarro L. Endothelium-derived nitric oxide: actions and properties. *The FASEB Journal.* 1989 January 1, 1989;3(1):31-6.
109. Behrendt D, Ganz P. Endothelial function. From vascular biology to clinical applications. *Am J Cardiol.* 2002;90(10C):40L-8L.
110. Dohi Y, Hahn AW, Boulanger CM, Buhler FR, Luscher TF. Endothelin stimulated by angiotensin II augments contractility of spontaneously hypertensive rat resistance arteries. *Hypertension.* 1992;19(2):131-7.
111. Taddei S, Favilla S, Duranti P, Simonini N, Salvetti A. Vascular renin-angiotensin system and neurotransmission in hypertensive persons. *Hypertension.* 1991;18(3):266-77.
112. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation.* 2007;115(10):1285-95.
113. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation.* 2006;113(13):1708-14.

114. Rhee SG. Cell signaling. H₂O₂, a necessary evil for cell signaling. *Science*. 2006;312(5782):1882-3.
115. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev*. 2002;23(5):599-622.
116. Nelson MR, Stepanek J, Cevette M, Covalciuc M, Hurst RT, Tajik AJ. Noninvasive measurement of central vascular pressures with arterial tonometry: clinical revival of the pulse pressure waveform? *Mayo*. 85(5):460-72.
117. Townsend RR. Analyzing the radial pulse waveform: narrowing the gap between blood pressure and outcomes. *Curr Opin Nephrol Hypertens*. 2007;16(3):261-6.
118. Sharman J, Stowasser M, Fassett R, Marwick T, Franklin S. Central blood pressure measurement may improve risk stratification. *J Hum Hypertens*. 2008;22(12):838-44. Epub 2008 Jul 3.
119. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation*. 2006;113(9):1213-25. Epub 2006 Feb 13.
120. Sharman JE, Fang ZY, Haluska B, Stowasser M, Prins JB, Marwick TH. Left ventricular mass in patients with type 2 diabetes is independently associated with central but not peripheral pulse pressure. *Diabetes Care*. 2005;28(4):937-9.
121. Weber T, Auer J, O'Rourke MF, Punzengruber C, Kvas E, Eber B. Prolonged mechanical systole and increased arterial wave reflections in diastolic dysfunction. *Heart*. 2006;92(11):1616-22. Epub 2006 May 18.
122. Lehmann ED, Hopkins KD, Rawesh A, Joseph RC, Kongola K, Coppack SW, et al. Relation between number of cardiovascular risk factors/events and noninvasive Doppler ultrasound assessments of aortic compliance. *Hypertension*. 1998;32(3):565-9.
123. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? *Circulation*. 2002;106(16):2085-90.
124. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension*. 2002;39(1):10-5.
125. Nabel EG, Selwyn AP, Ganz P. Large coronary arteries in humans are responsive to changing blood flow: an endothelium-dependent mechanism that fails in patients with atherosclerosis. *J Am Coll Cardiol*. 1990;16(2):349-56.
126. Joannides R, Bellien J, Thuillez C. Clinical methods for the evaluation of endothelial function-- a focus on resistance arteries. *Fundam Clin Pharmacol*. 2006;20(3):311-20.
127. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340(8828):1111-5.
128. Farouque HM, Meredith IT. The assessment of endothelial function in humans. *Coron Artery Dis*. 2001;12(6):445-54.
129. Sorensen KE, Celermajer DS, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Thomas O, et al. Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. *Br Heart J*. 1995;74(3):247-53.

130. Hardie KL, Kinlay S, Hardy DB, Wlodarczyk J, Silberberg JS, Fletcher PJ. Reproducibility of brachial ultrasonography and flow-mediated dilatation (FMD) for assessing endothelial function. *Aust N Z J Med*. 1997;27(6):649-52.
131. Shimbo D, Grahame-Clarke C, Miyake Y, Rodriguez C, Sciacca R, Di Tullio M, et al. The association between endothelial dysfunction and cardiovascular outcomes in a population-based multi-ethnic cohort. *Atherosclerosis*. 2007;192(1):197-203. Epub 2006 Jun 8.
132. Yeboah J, Burke GL, Crouse JR, Herrington DM. Relationship between brachial flow-mediated dilation and carotid intima-media thickness in an elderly cohort: the Cardiovascular Health Study. *Atherosclerosis*. 2008;197(2):840-5. Epub 2007 Sep 4.
133. Shechter M, Marai I, Marai S, Sherer Y, Sela BA, Feinberg MS, et al. The association of endothelial dysfunction and cardiovascular events in healthy subjects and patients with cardiovascular disease. *Isr Med Assoc J*. 2007;9(4):271-6.
134. Rossi R, Nuzzo A, Origliani G, Modena MG. Prognostic Role of Flow-Mediated Dilation and Cardiac Risk Factors in Post-Menopausal Women. *J Am Coll Cardiol*. 2008 March 11, 2008;51(10):997-1002.
135. Suessenbacher A, Frick M, Alber HF, Barbieri V, Pachinger O, Weidinger F. Association of improvement of brachial artery flow-mediated vasodilation with cardiovascular events. *Vasc Med*. 2006;11(4):239-44.
136. Verdecchia P, Reboldi G, Schillaci G, Borgioni C, Ciucci A, Telera MP, et al. Circulating Insulin and Insulin Growth Factor-1 Are Independent Determinants of Left Ventricular Mass and Geometry in Essential Hypertension. *Circulation*. 1999 October 26, 1999;100(17):1802-7.
137. Hirayama H, Sugano M, Abe N, Yonemochi H, Makino N. Determination of left ventricular mass by echocardiography in normotensive diabetic patients. *Jpn Circ J*. 2000;64(12):921-4.
138. Sundstrom J, Lind L, Nystrom N, Zethelius B, Andren B, Hales CN, et al. Left Ventricular Concentric Remodeling Rather Than Left Ventricular Hypertrophy Is Related to the Insulin Resistance Syndrome in Elderly Men. *Circulation*. 2000 June 6, 2000;101(22):2595-600.
139. Palmieri V, Bella JN, Arnett DK, Liu JE, Oberman A, Schuck M-Y, et al. Effect of Type 2 Diabetes Mellitus on Left Ventricular Geometry and Systolic Function in Hypertensive Subjects : Hypertension Genetic Epidemiology Network (HyperGEN) Study. *Circulation*. 2001 January 2, 2001;103(1):102-7.
140. Galvan AQ, Galetta F, Natali A, Muscelli E, Sironi AM, Cini G, et al. Insulin Resistance and Hyperinsulinemia : No Independent Relation to Left Ventricular Mass in Humans. *Circulation*. 2000 October 31, 2000;102(18):2233-8.
141. Uusitupa M, Siitonen O, Pyorala K, Mustonen J, Voutilainen E, Hersio K, et al. Relationship of blood pressure and left ventricular mass to serum insulin levels in newly diagnosed non-insulin-dependent (type 2) diabetic patients and in non-diabetic subjects. *Diabetes Res*. 1987;4(1):19-25.
142. Young ME, McNulty P, Taegtmeier H. Adaptation and Maladaptation of the Heart in Diabetes: Part II: Potential Mechanisms. *Circulation*. 2002 April 16, 2002;105(15):1861-70.
143. Hill DJ, Milner RD. Insulin as a growth factor. *Pediatr Res*. 1985;19(9):879-86.
144. Ruiz-Torres A, Melon J, Munoz FJ. Insulin stimulates collagen synthesis in vascular smooth muscle cells from elderly patients. *Gerontology*. 1998;44(3):144-8.

145. Landsberg L, Krieger DR. Obesity, metabolism, and the sympathetic nervous system. *Am J Hypertens*. 1989;2(3 Pt 2):125S-32S.
146. Fraser R. Studying genes and the development of cardiac hypertrophy: convenient intermediate phenotypes in man. *J Hypertens*. 2003;21(5):873-4.
147. Deschepper CF, Boutin-Ganache I, Zahabi A, Jiang Z. In search of cardiovascular candidate genes: interactions between phenotypes and genotypes. *Hypertension*. 2002;39(2 Pt 2):332-6.
148. Shigematsu Y, Hara Y, Ohtsuka T, Ohgimoto A, Inoue K, Higaki J. Relation of genetic predisposition and insulin resistance to left ventricular hypertrophy in hypertension. *Am J Hypertens*. 2005;18(4):457-63.
149. Hong KW, Shin DJ, Lee SH, Son NH, Go MJ, Lim JE, et al. Common variants in RYR1 are associated with left ventricular hypertrophy assessed by electrocardiogram. *Eur Heart J*. 2012;33(10):1250-6. doi: 10.093/eurheartj/ehr267. Epub 2011 Aug 9.
150. Everett AD, Tufro-McReddie A, Fisher A, Gomez RA. Angiotensin receptor regulates cardiac hypertrophy and transforming growth factor-beta 1 expression. *Hypertension*. 1994;23(5):587-92.
151. Schmieder RE, Langenfeld MR, Friedrich A, Schobel HP, Gatzka CD, Weihprecht H. Angiotensin II related to sodium excretion modulates left ventricular structure in human essential hypertension. *Circulation*. 1996;94(6):1304-9.
152. Klingbeil AU, Schobel H, Langenfeld MR, Hilgers K, Schaufele T, Schmieder RE. Hyper-responsiveness to angiotensin II is related to cardiac structural adaptation in hypertensive subjects. *J Hypertens*. 1999;17(6):825-33.
153. Sudoh T, Kangawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. *Nature*. 1988;332(6159):78-81.
154. Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation*. 1994;90(1):195-203.
155. Sayama H, Nakamura Y, Saito N, Kinoshita M. Why is the concentration of plasma brain natriuretic peptide in elderly inpatients greater than normal? *Coron Artery Dis*. 1999;10(7):537-40.
156. Nishikimi T, Yoshihara F, Morimoto A, Ishikawa K, Ishimitsu T, Saito Y, et al. Relationship between left ventricular geometry and natriuretic peptide levels in essential hypertension. *Hypertension*. 1996;28(1):22-30.
157. Kohno M, Horio T, Yokokawa K, Murakawa K, Yasunari K, Akioka K, et al. Brain natriuretic peptide as a cardiac hormone in essential hypertension. *Am J Med*. 1992;92(1):29-34.
158. Luchner A, Burnett JC, Jr., Jougasaki M, Hense HW, Heid IM, Muders F, et al. Evaluation of brain natriuretic peptide as marker of left ventricular dysfunction and hypertrophy in the population. *J Hypertens*. 2000;18(8):1121-8.
159. Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Gattobigio R, Zampi I, et al. Prognostic significance of serial changes in left ventricular mass in essential hypertension. *Circulation*. 1998;97(1):48-54.
160. Koren MJ, Ulin RJ, Koren AT, Laragh JH, Devereux RB. Left ventricular mass change during treatment and outcome in patients with essential hypertension. *Am J Hypertens*. 2002;15(12):1021-8.
161. Verdecchia P, Angeli F, Borgioni C, Gattobigio R, de Simone G, Devereux RB, et al. Changes in cardiovascular risk by reduction of left ventricular mass in hypertension: a meta-analysis. *Am J Hypertens*. 2003;16(11 Pt 1):895-9.

162. Pierdomenico SD, Lapenna D, Cuccurullo F. Regression of echocardiographic left ventricular hypertrophy after 2 years of therapy reduces cardiovascular risk in patients with essential hypertension. *Am J Hypertens*. 2008;21(4):464-70. Epub 2008 Feb 7.
163. Dahlöf B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet*. 2002;359(9311):995-1003.
164. Okin PM, Devereux RB, Jern S, Kjeldsen SE, Julius S, Nieminen MS, et al. Regression of electrocardiographic left ventricular hypertrophy by losartan versus atenolol: The Losartan Intervention for Endpoint reduction in Hypertension (LIFE) Study. *Circulation*. 2003;108(6):684-90. Epub 2003 Jul 28.
165. Devereux RB, Dahlöf B, Gerds E, Boman K, Nieminen MS, Papademetriou V, et al. Regression of hypertensive left ventricular hypertrophy by losartan compared with atenolol: the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) trial. *Circulation*. 2004;110(11):1456-62. Epub 2004 Aug 23.
166. Okin PM, Devereux RB, Harris KE, Jern S, Kjeldsen SE, Lindholm LH, et al. In-treatment resolution or absence of electrocardiographic left ventricular hypertrophy is associated with decreased incidence of new-onset diabetes mellitus in hypertensive patients: the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) Study. *Hypertension*. 2007;50(5):984-90. Epub 2007 Sep 24.
167. Lindholm LH, Ibsen H, Dahlöf B, Devereux RB, Beevers G, de Faire U, et al. Cardiovascular morbidity and mortality in patients with diabetes in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet*. 2002;359(9311):1004-10.
168. Okin PM, Devereux RB, Gerds E, Snapinn SM, Harris KE, Jern S, et al. Impact of diabetes mellitus on regression of electrocardiographic left ventricular hypertrophy and the prediction of outcome during antihypertensive therapy: the Losartan Intervention For Endpoint (LIFE) Reduction in Hypertension Study. *Circulation*. 2006;113(12):1588-96. Epub 2006 Mar 13.
169. Okin PM, Devereux RB, Harris KE, Jern S, Kjeldsen SE, Julius S, et al. Regression of electrocardiographic left ventricular hypertrophy is associated with less hospitalization for heart failure in hypertensive patients. *Ann Intern Med*. 2007;147(5):311-9.
170. Wachtell K, Okin PM, Olsen MH, Dahlöf B, Devereux RB, Ibsen H, et al. Regression of electrocardiographic left ventricular hypertrophy during antihypertensive therapy and reduction in sudden cardiac death: the LIFE Study. *Circulation*. 2007;116(7):700-5. Epub 2007 Jul 30.
171. Okin PM, Wachtell K, Devereux RB, Harris KE, Jern S, Kjeldsen SE, et al. Regression of electrocardiographic left ventricular hypertrophy and decreased incidence of new-onset atrial fibrillation in patients with hypertension. *Jama*. 2006;296(10):1242-8.
172. Okin PM, Devereux RB, Jern S, Kjeldsen SE, Julius S, Nieminen MS, et al. Regression of electrocardiographic left ventricular hypertrophy during antihypertensive treatment and the prediction of major cardiovascular events. *Jama*. 2004;292(19):2343-9.
173. Hoiegggen A, Alderman MH, Kjeldsen SE, Julius S, Devereux RB, De Faire U, et al. The impact of serum uric acid on cardiovascular outcomes in the LIFE study. *Kidney Int*. 2004;65(3):1041-9.

174. Effects of an Angiotensin-Converting Enzyme Inhibitor, Ramipril, on Cardiovascular Events in High-Risk Patients. *New England Journal of Medicine*. 2000;342(3):145-53.
175. Mathew J, Sleight P, Lonn E, Johnstone D, Pogue J, Yi Q, et al. Reduction of cardiovascular risk by regression of electrocardiographic markers of left ventricular hypertrophy by the angiotensin-converting enzyme inhibitor ramipril. *Circulation*. 2001;104(14):1615-21.
176. Lonn E, Shaikholeslami R, Yi Q, Bosch J, Sullivan B, Tanser P, et al. Effects of ramipril on left ventricular mass and function in cardiovascular patients with controlled blood pressure and with preserved left ventricular ejection fraction: a substudy of the Heart Outcomes Prevention Evaluation (HOPE) Trial. *J Am Coll Cardiol*. 2004;43(12):2200-6.
177. Nielsen FS, Sato A, Ali S, Tarnow L, Smidt UM, Kastrup J, et al. Beneficial impact of ramipril on left ventricular hypertrophy in normotensive nonalbuminuric NIDDM patients. *Diabetes Care*. 1998;21(5):804-9.
178. Liebson PR, Grandits GA, Dianzumba S, Prineas RJ, Grimm RH, Jr., Neaton JD, et al. Comparison of five antihypertensive monotherapies and placebo for change in left ventricular mass in patients receiving nutritional-hygienic therapy in the Treatment of Mild Hypertension Study (TOMHS). *Circulation*. 1995;91(3):698-706.
179. Jula AM, Karanko HM. Effects on left ventricular hypertrophy of long-term nonpharmacological treatment with sodium restriction in mild-to-moderate essential hypertension. *Circulation*. 1994;89(3):1023-31.
180. Mancia G, Carugo S, Grassi G, Lanzarotti A, Schiavina R, Cesana G, et al. Prevalence of Left Ventricular Hypertrophy in Hypertensive Patients Without and With Blood Pressure Control: Data From the PAMELA Population. *Hypertension*. 2002 March 1, 2002;39(3):744-9.
181. Brown DW, Giles WH, Croft JB. Left ventricular hypertrophy as a predictor of coronary heart disease mortality and the effect of hypertension. *American Heart Journal*. 2000;140(6):848-56.
182. 56 BNF. September 2008.
183. Graham S, Day RO, Wong H, McLachlan AJ, Bergendal L, Miners JO, et al. Pharmacodynamics of oxypurinol after administration of allopurinol to healthy subjects. *Br J Clin Pharmacol*. 1996 Apr;41(4):299-304.
184. Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev*. 2006 Mar;58(1):87-114.
185. http://nobelprize.org/nobel_prizes/medicine/laureates/1988/elion-lecture.pdf.
186. George J, Struthers AD. The role of urate and xanthine oxidase inhibitors in cardiovascular disease. *Cardiovasc Ther*. 2008 Spring;26(1):59-64.
187. Harrison R. Structure and function of xanthine oxidoreductase: where are we now? *Free Radic Biol Med*. 2002 Sep 15;33(6):774-97.
188. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J Physiol*. 2004 March 15, 2004;555(3):589-606.
189. Xia M, Dempski R, Hille R. The reductive half-reaction of xanthine oxidase. Reaction with aldehyde substrates and identification of the catalytically labile oxygen. *J Biol Chem*. 1999 Feb 5;274(6):3323-30.
190. Hille R, Massey V. Studies on the oxidative half-reaction of xanthine oxidase. *J Biol Chem*. 1981 Sep 10;256(17):9090-5.

191. Parks DA, Granger DN. Xanthine oxidase: biochemistry, distribution and physiology. *Acta Physiol Scand Suppl.* 1986;548:87-99.
192. Krenitsky TA, Tuttle JV, Cattau EL, Jr., Wang P. A comparison of the distribution and electron acceptor specificities of xanthine oxidase and aldehyde oxidase. *Comp Biochem Physiol B.* 1974 Dec 15;49(4):687-703.
193. Muxfeldt M, Schaper W. The activity of xanthine oxidase in heart of pigs, guinea pigs, rabbits, rats, and humans. *Basic Res Cardiol.* 1987 Sep-Oct;82(5):486-92.
194. Bruder G, Heid HW, Jarasch ED, Mather IH. Immunological identification and determination of xanthine oxidase in cells and tissues. *Differentiation.* 1983;23(3):218-25.
195. Battelli MG, Musiani S, Valgimigli M, Gramantieri L, Tomassoni F, Bolondi L, et al. Serum xanthine oxidase in human liver disease. *Am J Gastroenterol.* 2001 Apr;96(4):1194-9.
196. Wolko K, Krawczynski J. Diagnostic value of determination of serum xanthine oxidase activity in acute viral hepatitis. *Mater Med Pol.* 1974 Apr-Jun;6(2):95-8.
197. Miesel R, Zuber M. Elevated levels of xanthine oxidase in serum of patients with inflammatory and autoimmune rheumatic diseases. *Inflammation.* 1993 Oct;17(5):551-61.
198. Radi R, Rubbo H, Bush K, Freeman BA. Xanthine oxidase binding to glycosaminoglycans: kinetics and superoxide dismutase interactions of immobilized xanthine oxidase-heparin complexes. *Arch Biochem Biophys.* 1997 Mar 1;339(1):125-35.
199. Panus PC, Wright SA, Chumley PH, Radi R, Freeman BA. The contribution of vascular endothelial xanthine dehydrogenase/oxidase to oxygen-mediated cell injury. *Arch Biochem Biophys.* 1992 May 1;294(2):695-702.
200. Paler-Martinez A, Panus PC, Chumley PH, Ryan U, Hardy MM, Freeman BA. Endogenous xanthine oxidase does not significantly contribute to vascular endothelial production of reactive oxygen species. *Arch Biochem Biophys.* 1994 May 15;311(1):79-85.
201. Kittleson MM, Hare JM. Xanthine oxidase inhibitors: an emerging class of drugs for heart failure. *Eur Heart J.* 2005 August 1, 2005;26(15):1458-60.
202. Granger DN, Rutili G, McCord JM. Superoxide radicals in feline intestinal ischemia. *Gastroenterology.* 1981 Jul;81(1):22-9.
203. Sisto T, Paajanen H, Metsa-Ketela T, Harmoinen A, Nordback I, Tarkka M. Pretreatment with antioxidants and allopurinol diminishes cardiac onset events in coronary artery bypass grafting. *Ann Thorac Surg.* 1995 Jun;59(6):1519-23.
204. Granger DN, Benoit JN, Suzuki M, Grisham MB. Leukocyte adherence to venular endothelium during ischemia-reperfusion. *Am J Physiol Gastrointest Liver Physiol.* 1989 November 1, 1989;257(5):G683-8.
205. Day RO, Graham GG, Hicks M, McLachlan AJ, Stocker SL, Williams KM. Clinical pharmacokinetics and pharmacodynamics of allopurinol and oxypurinol. *Clin Pharmacokinet.* 2007;46(8):623-44.
206. Murrell GA, Rapeport WG. Clinical pharmacokinetics of allopurinol. *Clin Pharmacokinet.* 1986 Sep-Oct;11(5):343-53.
207. Appelbaum SJ, Mayersohn M, Dorr RT, Perrier D. Allopurinol kinetics and bioavailability. Intravenous, oral and rectal administration. *Cancer Chemother Pharmacol.* 1982;8(1):93-8.

208. Breithaupt B, Tittel M. Kinetics of allopurinol after single intravenous and oral doses. Noninteraction with benzbromarone and hydrochlorothiazide. *Eur J Clin Pharmacol.* 1982;22(1):77-84.
209. Turnheim K, Krivanek P, Oberbauer R. Pharmacokinetics and pharmacodynamics of allopurinol in elderly and young subjects. *Br J Clin Pharmacol.* 1999 Oct;48(4):501-9.
210. Pea F. Pharmacology of drugs for hyperuricemia. Mechanisms, kinetics and interactions. *Contrib Nephrol.* 2005;147:35-46.
211. George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. *Circulation.* 2006 Dec 5;114(23):2508-16.
212. Butler R, Morris AD, Belch JJ, Hill A, Struthers AD. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension.* 2000 Mar;35(3):746-51.
213. Guan W, Osanai T, Kamada T, Hanada H, Ishizaka H, Onodera H, et al. Effect of allopurinol pretreatment on free radical generation after primary coronary angioplasty for acute myocardial infarction. *J Cardiovasc Pharmacol.* 2003 May;41(5):699-705.
214. Farquharson CA, Butler R, Hill A, Belch JJ, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. *Circulation.* 2002 Jul 9;106(2):221-6.
215. Guthikonda S, Sinkey C, Barenz T, Haynes WG. Xanthine oxidase inhibition reverses endothelial dysfunction in heavy smokers. *Circulation.* 2003 Jan 28;107(3):416-21.
216. Ekelund UEG, Harrison RW, Shokek O, Thakkar RN, Tunin RS, Senzaki H, et al. Intravenous Allopurinol Decreases Myocardial Oxygen Consumption and Increases Mechanical Efficiency in Dogs With Pacing-Induced Heart Failure. *Circ Res.* 1999 September 3, 1999;85(5):437-45.
217. Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, et al. Allopurinol Improves Myocardial Efficiency in Patients With Idiopathic Dilated Cardiomyopathy. *Circulation.* 2001 November 13, 2001;104(20):2407-11.
218. Das DK, Engelman RM, Clement R, Otani H, Prasad MR, Rao PS. Role of xanthine oxidase inhibitor as free radical scavenger: A novel mechanism of action of allopurinol and oxypurinol in myocardial salvage. *Biochemical and Biophysical Research Communications.* 1987;148(1):314-9.
219. Hoey BM, Butler J, Halliwell B. On the specificity of allopurinol and oxypurinol as inhibitors of xanthine oxidase. A pulse radiolysis determination of rate constants for reaction of allopurinol and oxypurinol with hydroxyl radicals. *Free Radic Res Commun.* 1988;4(4):259-63.
220. Malkiel S, Har-el R, Schwalb H, Uretzky G, Borman JB, Chevion M. Interaction between allopurinol and copper: possible role in myocardial protection. *Free Radic Res Commun.* 1993;18(1):7-15.
221. Johnson WD, Kayser KL, Brenowitz JB, Saedi SF. A randomized controlled trial of allopurinol in coronary bypass surgery. *Am Heart J.* 1991 Jan;121(1 Pt 1):20-4.
222. Movahed A, Nair KG, Ashavaid TF, Kumar P. Free radical generation and the role of allopurinol as a cardioprotective agent during coronary artery bypass grafting surgery. *Can J Cardiol.* 1996 Feb;12(2):138-44.

223. Belboul A, Roberts D, Borjesson R, Johnsson J. Oxygen free radical generation in healthy blood donors and cardiac patients: the protective effect of allopurinol. *Perfusion*. 2001 Jan;16(1):59-65.
224. Nishizawa J, Nakai A, Matsuda K, Komeda M, Ban T, Nagata K. Reactive Oxygen Species Play an Important Role in the Activation of Heat Shock Factor 1 in Ischemic-Reperfused Heart. *Circulation*. 1999 February 23, 1999;99(7):934-41.
225. Engberding N, Spiekermann S, Schaefer A, Heineke A, Wiencke A, Muller M, et al. Allopurinol Attenuates Left Ventricular Remodeling and Dysfunction After Experimental Myocardial Infarction: A New Action for an Old Drug? *Circulation*. 2004 October 12, 2004;110(15):2175-9.
226. Kinugawa S, Tsutsui H, Hayashidani S, Ide T, Suematsu N, Satoh S, et al. Treatment With Dimethylthiourea Prevents Left Ventricular Remodeling and Failure After Experimental Myocardial Infarction in Mice : Role of Oxidative Stress. *Circ Res*. 2000 September 1, 2000;87(5):392-8.
227. Rajesh M, Mukhopadhyay P, Batkai S, Mukhopadhyay B, Patel V, Hasko G, et al. Xanthine oxidase inhibitor allopurinol attenuates the development of diabetic cardiomyopathy. *J Cell Mol Med*. 2008 Oct 23;23:23.
228. Struthers AD, Donnan PT, Lindsay P, McNaughton D, Broomhall J, MacDonald TM. Effect of allopurinol on mortality and hospitalisations in chronic heart failure: a retrospective cohort study. *Heart*. 2002 March 1, 2002;87(3):229-34.
229. Doehner W, Schoene N, Rauchhaus M, Leyva-Leon F, Pavitt DV, Reaveley DA, et al. Effects of Xanthine Oxidase Inhibition With Allopurinol on Endothelial Function and Peripheral Blood Flow in Hyperuricemic Patients With Chronic Heart Failure: Results From 2 Placebo-Controlled Studies. *Circulation*. 2002 June 4, 2002;105(22):2619-24.
230. El Solh AA, Saliba R, Bosinski T, Grant BJB, Berbary E, Miller N. Allopurinol improves endothelial function in sleep apnoea: a randomised controlled study. *Eur Respir J*. 2006 May 1, 2006;27(5):997-1002.
231. Khan F, George J, Wong K, McSwiggan S, Struthers AD, Belch JJ. Allopurinol treatment reduces arterial wave reflection in stroke survivors. *Cardiovasc Ther*. 2008;26(4):247-52.
232. Gavin AD, Struthers AD. Allopurinol reduces B-type natriuretic peptide concentrations and haemoglobin but does not alter exercise capacity in chronic heart failure. *Heart*. 2005;91(6):749-53.
233. Dawson J, Lees KR, Weir CJ, Quinn T, Ali M, Hennerici MG, et al. Baseline serum urate and 90-day functional outcomes following acute ischemic stroke. *Cerebrovasc Dis*. 2009;28(2):202-3. doi: 10.1159/000226580. Epub 2009 Jun 30.
234. Muir SW, Harrow C, Dawson J, Lees KR, Weir CJ, Sattar N, et al. Allopurinol use yields potentially beneficial effects on inflammatory indices in those with recent ischemic stroke: a randomized, double-blind, placebo-controlled trial. *Stroke*. 2008;39(12):3303-7. doi: 10.1161/STROKEAHA.108.519793. Epub 2008 Oct 9.
235. Dawson J, Quinn TJ, Harrow C, Lees KR, Walters MR. The effect of allopurinol on the cerebral vasculature of patients with subcortical stroke; a randomized trial. *Br J Clin Pharmacol*. 2009;68(5):662-8. doi: 10.1111/j.365-2125.009.03497.x.
236. Kao MP, Ang DS, Gandy SJ, Nadir MA, Houston JG, Lang CC, et al. Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease. *J Am Soc Nephrol*. 2011;22(7):1382-9. Epub 2011 Jun 30.

237. Feig DI, Soletsky B, Johnson RJ. Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. *Jama*. 2008;300(8):924-32.
238. Kanbay M, Ozkara A, Selcoki Y, Isik B, Turgut F, Bavbek N, et al. Effect of treatment of hyperuricemia with allopurinol on blood pressure, creatinine clearance, and proteinuria in patients with normal renal functions. *Int Urol Nephrol*. 2007;39(4):1227-33. Epub 2007 Aug 15.
239. Agarwal V, Hans N, Messerli FH. Effect of allopurinol on blood pressure: a systematic review and meta-analysis. *J Clin Hypertens (Greenwich)*. 2013;15(6):435-42. doi: 10.1111/j.751-7176.2012.00701.x. Epub 2012 Aug 20.
240. Loeffler LF, Navas-Acien A, Brady TM, Miller ER, 3rd, Fadrowski JJ. Uric acid level and elevated blood pressure in US adolescents: National Health and Nutrition Examination Survey, 1999-2006. *Hypertension*. 2012;59(4):811-7. Epub 2012 Feb 21.
241. Cingolani HE, Plastino JA, Escudero EM, Mangal B, Brown J, Perez NG. The effect of xanthine oxidase inhibition upon ejection fraction in heart failure patients: La Plata Study. *J Card Fail*. 2006;12(7):491-8.
242. Rekhraj S, Gandy SJ, Szwejkowski BR, Nadir MA, Noman A, Houston JG, et al. High-dose allopurinol reduces left ventricular mass in patients with ischemic heart disease. *J Am Coll Cardiol*. 2013;61(9):926-32. doi: 10.1016/j.jacc.2012.09.066.
243. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005 Dec;18(12):1440-63.
244. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002 Jan 16;39(2):257-65.
245. Rajendra NS, Ireland S, George J, Belch JJ, Lang CC, Struthers AD. Mechanistic insights into the therapeutic use of high-dose allopurinol in angina pectoris. *J Am Coll Cardiol*. 2011 Aug 16;58(8):820-8.
246. Myerson SG, Bellenger NG, Pennell DJ. Assessment of left ventricular mass by cardiovascular magnetic resonance. *Hypertension*. 2002 Mar 1;39(3):750-5.
247. Grothues F, Smith GC, Moon JC, Bellenger NG, Collins P, Klein HU, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol*. 2002;90(1):29-34.
248. Simpson HJ, Gandy SJ, Houston JG, Rajendra NS, Davies JJ, Struthers AD. Left ventricular hypertrophy: reduction of blood pressure already in the normal range further regresses left ventricular mass. *Heart*. 2010;96(2):148-52. Epub 2009 Oct 26.
249. Schillaci G. Pharmacogenomics of left ventricular hypertrophy reversal: beyond the 'one size fits all' approach to antihypertensive therapy. *J Hypertens*. 2004;22(12):2273-5.
250. Devereux RB, Wachtell K, Gerdts E, Boman K, Nieminen MS, Papademetriou V, et al. Prognostic Significance of Left Ventricular Mass Change During Treatment of Hypertension. *JAMA*. 2004 November 17, 2004;292(19):2350-6.

251. Laakso JT, Teravainen TL, Martelin E, Vaskonen T, Lapatto R. Renal xanthine oxidoreductase activity during development of hypertension in spontaneously hypertensive rats. *J Hypertens*. 2004;22(7):1333-40.
252. Xu X, Hu X, Lu Z, Zhang P, Zhao L, Wessale JL, et al. Xanthine oxidase inhibition with febuxostat attenuates systolic overload-induced left ventricular hypertrophy and dysfunction in mice. *J Card Fail*. 2008;14(9):746-53. Epub 2008 Jul 10.
253. Ogino K, Kato M, Furuse Y, Kinugasa Y, Ishida K, Osaki S, et al. Uric acid-lowering treatment with benzbromarone in patients with heart failure: a double-blind placebo-controlled crossover preliminary study. *Circ Heart Fail*. 2010;3(1):73-81. Epub 2009 Nov 20.
254. Lee MH, Graham GG, Williams KM, Day RO. A benefit-risk assessment of benzbromarone in the treatment of gout. Was its withdrawal from the market in the best interest of patients? *Drug Saf*. 2008;31(8):643-65.
255. Hirsch GA, Bottomley PA, Gerstenblith G, Weiss RG. Allopurinol acutely increases adenosine triphosphate energy delivery in failing human hearts. *J Am Coll Cardiol*. 2012;59(9):802-8.
256. Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic cardiomyopathy: a paradigm for myocardial energy depletion. *Trends Genet*. 2003;19(5):263-8.
257. Naumova AV, Chacko VP, Ouwerkerk R, Stull L, Marban E, Weiss RG. Xanthine oxidase inhibitors improve energetics and function after infarction in failing mouse hearts. *Am J Physiol Heart Circ Physiol*. 2006;290(2):H837-43. Epub 2005 Sep 23.
258. Zhao L, Roche BM, Wessale JL, Kijawornrat A, Lolly JL, Shemanski D, et al. Chronic xanthine oxidase inhibition following myocardial infarction in rabbits: effects of early versus delayed treatment. *Life Sci*. 2008;82(9-10):495-502. Epub 2008 Jan 24.
259. Mellin V, Isabelle M, Oudot A, Vergely-Vandriesse C, Monteil C, Di Meglio B, et al. Transient reduction in myocardial free oxygen radical levels is involved in the improved cardiac function and structure after long-term allopurinol treatment initiated in established chronic heart failure. *Eur Heart J*. 2005;26(15):1544-50. Epub 2005 May 4.
260. Verdecchia P, Schillaci G, Reboldi G, Santeusano F, Porcellati C, Brunetti P. Relation between serum uric acid and risk of cardiovascular disease in essential hypertension. The PIUMA study. *Hypertension*. 2000;36(6):1072-8.
261. Viazzi F, Parodi D, Leoncini G, Parodi A, Falqui V, Ratto E, et al. Serum uric acid and target organ damage in primary hypertension. *Hypertension*. 2005;45(5):991-6. Epub 2005 Mar 21.
262. Mitsuhashi H, Yatsuya H, Matsushita K, Zhang H, Otsuka R, Muramatsu T, et al. Uric acid and left ventricular hypertrophy in Japanese men. *Circ J*. 2009;73(4):667-72. Epub 2009 Feb 19.
263. Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, et al. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension*. 2003;41(6):1183-90. Epub 2003 Apr 21.
264. McInnes GT, Lawson DH, Jick H. Acute adverse reactions attributed to allopurinol in hospitalised patients. *Ann Rheum Dis*. 1981;40(3):245-9.
265. Wei L, Fahey T, Struthers AD, MacDonald TM. Association between allopurinol and mortality in heart failure patients: a long-term follow-up study. *Int J Clin Pract*. 2009;63(9):1327-33. doi: 10.1111/j.742-241.2009.02118.x.

18. Appendices

Consent form

Patient Information Sheet

GP Letter

Case Report Form



Division of Medicine and Therapeutics

Head of Division and Professor of Cardiovascular Medicine and Therapeutics
Professor Allan D Struthers BSc MD FRCP FESC

25/09/2009
 Version 3.0

Study Number: eb/lm/let390/ln950/20038

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: Do Xanthine Oxidase Inhibitors reduce both Left Ventricular Hypertrophy and Endothelial Dysfunction in Cardiovascular Patients with Diabetes?

Name of Researcher: Dr Ben Szwejkowski

Please initial box

1. I confirm that I have read and understood the information sheet dated 24/9/09 (version 5) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without any medical care or legal rights being affected ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from NHS Tayside, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree to my GP being informed of my participation in the study. ☐
5. I agree to any tissue (blood samples) taken during the study being retained for use in future studies. ☐
6. I agree to have an x-ray of my eyes if required prior to undergoing an MRI scan for safety reasons. ☐
7. I agree to take part in the above study. ☐

 Name of participant

 Date

 Signature

 Name of person taking consent

 Date

 Signature

When complete, 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes



Division of Medicine and Therapeutics

Head of Division and Professor of Cardiovascular Medicine and Therapeutics
Professor Allan D Struthers BSc MD FRCP FESC

12/01/2009

Version 6.0

Do Xanthine Oxidase Inhibitors Reduce Left Ventricular Hypertrophy in Diabetic Patients?

PATIENT INFORMATION SHEET

You are being asked to participate in a research project. This research will also be used in part towards an educational qualification with the University of Dundee. Before you decide, it is important for you to understand why the research is being carried out and what it involves. Please take time to read the following information carefully and discuss it with others, if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time decide whether or not you would like to take part.

Background

In patients with diabetes, “left ventricular hypertrophy” or thickened heart muscle is a very common occurrence. Thickened heart muscle is diagnosed using an ultrasound scan of the heart (also known as echocardiography) and this has been shown to adversely affect one’s long term prognosis (symptoms and survival). Patients with thickened heart muscle are also known to have thick blood vessels and more prone to life threatening abnormal heart beats. Studies have shown that if we can reverse thickened heart muscle and stiffened blood vessels we can substantially reduce the long term complications associated with these conditions. Recently it has been found that a tablet called allopurinol, a drug commonly used in the treatment of gout for many years, has beneficial effects that may reduce thickened heart muscle, stiffened blood vessels and the chance of life threatening irregular heart beats. Thickened heart muscle will be evaluated with Magnetic Resonance Imaging (MRI).

What are we asking you to do?

The study takes 9 months to complete. The study is of a randomised, double blind design. This means that you will take a tablet, which will be either allopurinol (the test drug) or a placebo (an inactive tablet). The tablet that you will be allocated is decided in a random way (a bit like tossing a coin), such that neither you nor the research team will know which tablet you are taking, until after the study is completed when a special randomisation code is opened. This means the results of the study cannot be influenced.

Firstly, we will ask you to visit us so that we can answer any questions that you may have. If you have not undergone an ultrasound scan of your heart before, we will perform this simple test during this visit to see if you have thickened heart muscles. After making sure that you are suitable to participate in the trial and obtaining your consent, we will also arrange a suitable date and time to perform an MRI scan to assess the structure of your heart. You will also be assigned to take either the test medication i.e. allopurinol or the placebo. During your subsequent visits to the department, we will also assess the stiffness of your blood vessels using ultrasound. Throughout the study duration, we will monitor your kidney function closely by means of a simple blood test (about 5 ml blood, or about a teaspoonful will be taken). Urine samples will also be collected during each visit to check for the amount of protein in the urine. Your blood pressure will also be measured during these visits. The rest of the study visits are outlined in the visit schedule (see attached sheet).

Blood samples will be stored and analysed at the end of the study. We will also store the blood samples for a period of 10 years after the end of the study, in case there are any new tests of blood vessel function or thickened heart muscle that become available after this time that we can analyse on your blood samples. Blood samples are not taken for genetic testing. These blood samples will be registered with the Tayside Tissue Bank

The tablets

Allopurinol is a tablet that has been in use for around 50 years for the treatment of gout. It has a good safety record and is generally well tolerated. However, like most medicines, allopurinol occasionally causes side effects, particularly in the period soon after it is first taken. The most common side effect is nausea and some abdominal discomfort which affects less than 5-10% of patients treated with allopurinol and the effects of which can be minimised by taking the tablets with food. The dose of Allopurinol used in this study has been tested before in previous studies undertaken at the University of Dundee where the dose you are being asked to take was found to be safe.

Allopurinol causes a skin rash in a small proportion of patients (about 1% or less) as a result of a type of allergy to the medication. This may be associated with fever, swollen glands, joint pain and unusual blistering or bleeding. Were any of these symptoms to develop, you should stop taking allopurinol immediately and contact the study doctor as soon as possible. You may also seek advice from your GP.

Reports of other side effects to allopurinol are very rare (e.g. affect less than 1 in 10,000 people) and it is not always clear if they are truly related to the treatment. The complete range of reported side effects is set out in a Patient Information Leaflet, a copy of which is attached for your information. This will be further discussed with you before you make a final decision about taking part in this study.

Placebo tablets are inactive and we do not expect you to experience any side effects at all whilst taking these. All your other medications should be taken as normal.

The MRI scan

The MRI scanner allows us to look in a detailed way at the heart without the use of radiation. Having an MRI involves lying flat for about 45 minutes. You have to go into a narrow tube during the scan, thus the scans are not suitable for people who are claustrophobic. The scanner takes pictures of your heart by the use of a large magnet. Hence, people who have any fragments of metal in their bodies may not be able to have these scans. If you have ever been exposed to metal fragments in your eyes it is sometimes necessary to x-ray the eyes. This is purely for your safety and involves minimal radiation exposure. The nurse will ask you more questions regarding this before the scan. If you would like to see the MRI scanner before you make up your mind, this can be arranged.

Assessing blood vessel stiffness using ultrasound

At each of these four visits, we will measure how one of the blood vessels in your arm functions, to check the stiffness of it. This technique is called Flow Mediated Dilatation, FMD. This is done using an ultrasound machine. To see how stiff the blood vessel is, we need to see how it responds to short period of having the circulation to the forearm blocked and then see how it responds to a substance called GTN that is used to treat angina. What does this involve?

You will be asked to lie on a couch for approximately 30 minutes. A blood pressure cuff will be placed below your elbow. We will then take an ultrasound measurement of the artery above the elbow. This involves placing a probe gently on the skin above the elbow. We will then inflate the blood pressure cuff to occlude the circulation to the forearm for a period of 5 minutes. After 5 minutes, we will release the cuff and take another measurement at the artery above the elbow. Similarly, a further measurement will be taken before and after giving GTN spray under your tongue.

In addition to using the ultrasound machine, you will also have the pressure of your pulse at your wrist, neck and groin measured in a technique called Sphygmocor. This is a very simple procedure, which involves placing a small probe gently on your skin above the arteries on your wrist, neck and groin. It only takes about 15 minutes and is completely painless.

What are the discomforts, risks and side effects?

The side effects of the tablets are listed above. Having blood taken can cause some mild bruising. GTN (that is used under the tongue when we ultrasound the artery) can cause very transient headache, which usually goes away quickly. When the blood pressure cuff is inflated, there can be a mild discomfort, which goes away quickly once it is deflated. Finally, some people may feel claustrophobic in the MRI scanner.

What are the benefits of taking part in the study?

As mentioned earlier, you have been diagnosed with diabetes. You are attending your own doctor and possibly, the specialist clinic in the hospital. You will be monitored closely and will be seen by a heart specialist for each of your visit as part of the study. Besides having those tests as mentioned earlier being carried out, your medications will be reviewed on a regular basis. These tests will give us more information about

the function of your heart, blood circulation and kidney function. If any of these investigations reveal any new abnormalities, we will refer you to a specialist clinic in Ninewells Hospital. The study itself may not directly benefit you, but the results of the study may change how physicians treat diabetic patients and thickened heart muscle, and this potentially will have an impact on the way future patients are treated with this condition.

What are my rights?

All data collected in the study will be coded and stored on a computer protected by a password available only to the researchers. The storage and processing of data is fully compliant with the guidelines of the Tayside Committee on Medical Research Ethics. If you wish, results of the study can be made available to you or your GP when the study is complete.

Participation in this study is entirely voluntary, and you are free to refuse to take part or to withdraw from the study at any time, without having to give a reason and without this affecting your future medical care or your relationship with medical staff looking after you. Some insurance companies consider that participation in medical research such as this is a “material fact”, which should be mentioned in any proposal for health-related insurance, or which could influence their judgement in consideration of claims made under existing policies. You should check that participation in this research does not affect any policy you might be thinking about taking out or any existing policy. We will provide a taxi or reasonable travel expenses for all of the visits.

Will the research influence the treatment I receive?

The research does not alter the treatment you receive. Your specialist will start and stop treatments as determined by your clinical condition.

What will happen to the information collected in the study and will my taking part in the study be kept confidential?

Identifiable information about you and the data collected during the study will be held by the Institute of Cardiovascular Research, Ninewells Hospital and Medical School. No one outside the research team will have access to any identifying information and all identifiable information and the data will be kept securely. Parts of the samples taken will be stored in a locked freezer for up to 10 years. The samples will only be handled by approved members of the laboratory. The samples will only be used for future research if it is deemed appropriate by the Ethics and Research Committee.

Will I continue to receive the medicine used on this study after it finishes?

There is no guarantee of this. The study is designed to give an indication of possible benefit from the medicine being tested and it may be some time, before we can be sure about how useful it actually is. Decisions about your continued treatment will be taken by your family doctor, who is responsible for writing your prescriptions, although he/she may seek the advice of a hospital specialist, if necessary. It is

therefore likely that you will revert to your pre-study medication, unless there are good reasons to change.

Who has reviewed the study?

This study has been reviewed by an NHS Research Ethics, which has the responsibility for scrutinising proposals for medical research on humans, in accordance with the requirements of the Clinical Trials Regulations. In this case, the reviewing Committee was the Fife & Forth Valley Research Ethics Committee who have raised no objections from the point of view of medical ethics.

We will contact your GP to inform him/her of your participation in the study, unless you ask us not to. If you are worried at any time about the research, or the results, or wish to discuss things generally in further detail, then please do not hesitate to contact:

Dr Ben Szwejkowski MBChB, MRCP (UK)

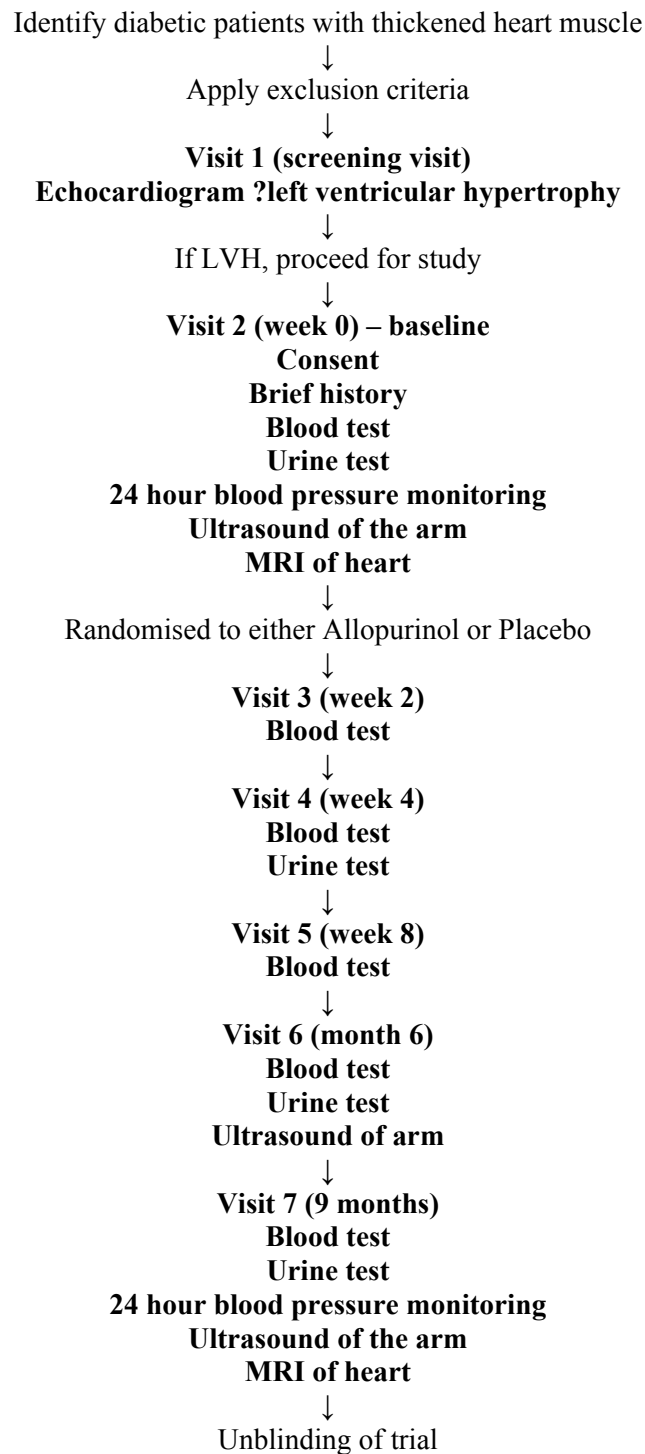
Specialty Registrar in Cardiology and Clinical Research Fellow in Cardiovascular Medicine

Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee DD1 9SY

Tel: (01382) 632180

e mail: b.szwejkowski@dundee.ac.uk

Visit Schedule



Thank You for taking time to read this Information Sheet and for considering taking part.



Division of Medicine and Therapeutics

Head of Division and Professor of Cardiovascular Medicine and Therapeutics
Professor Allan D Struthers BSc MD FRCP FESC

12/01/2010
Version 4.0

Dear Doctor,

RE:

Your patient has been asked to take part in a clinical trial entitled “Do Xanthine Oxidase Inhibitors Reduce Both Left Ventricular Hypertrophy and Vascular Dysfunction in Diabetic Patients”. This project is funded by Diabetes UK and will be carried out at the University of Dundee. The primary endpoint is to assess if Allopurinol reduces left ventricular hypertrophy in patients with diabetes. The secondary endpoint is to assess if Allopurinol improves endothelial function in diabetic patients.

This is a randomised, double blind placebo controlled trial using Allopurinol at a maximal dose of 600mg. They will undergo cardiac MRI at the beginning of the trial and at 9 months. They will also periodically have Flow Mediated Dilatation studies.

A detailed information sheet has been given to the patient. I would also ask you to contact myself directly if you feel the patient develops side effects from Allopurinol or has any problems finishing the study. I have outlined the study protocol for your information and attached it to this letter.

Yours sincerely,

Dr Ben Szwejkowski MBChB MRCP (UK)
 Cardiology Specialty Registrar, West of Scotland
 Cardiovascular Clinical Research Fellow, University of Dundee
 Department of Clinical Pharmacology, Ninewells Hospital and Medical School,
 Dundee DD1 9SY
 Tel: (01382) 632180 or (01382) 660111 extension 40097
 e mail: b.szwejkowski@dundee.ac.uk

Protocol “Do Xanthine Oxidase Inhibitors Reduce Both Left Ventricular Hypertrophy and Vascular Dysfunction in Diabetic patients”

Visit 1 screening visit

Interested patients will be screened by echocardiography to look for LVH, thickened heart muscle. If LVH is present they will be recruited to the study. The criteria for LVH will be American Society of Echocardiography (ASE) criteria of 115 gm for men and 95 gm women.

Visit 2 (week 0)

Informed consent

Baseline blood tests urea and electrolytes (U and E)
 Liver function tests
 Full blood count
 Uric acid
 Fasting glucose/insulin (HOMA index)
 HbA1c
 BNP
 PIIINP (collagen marker)
 Renin and aldosterone

Urine test for protein concentration

Sphygmocor to measure augmentation index (involves a small probe on the wrist, neck and groin to assess the character of the pulse)

Flow mediated dilatation (FMD) (ultrasound of the brachial artery to assess blood flow response)

Cardiac MRI

24 hour blood pressure monitoring

Visit 3 (week 2)

Blood test U and E only

Visit 4 (week 4)

Blood tests

Urine test

Visit 5 (week 8)

Blood tests

Urine test

Visit 6 (month 6)

Blood tests

Urine test

Sphygmocor

FMD

Visit 7 (month 6)

Blood tests

Urine test for protein concentration

Sphygmocor

FMD

Cardiac MRI

24 hour blood pressure monitoring



CASE REPORT FORM

Do Xanthine Oxidase Inhibitors Regress Left Ventricular Hypertrophy in Diabetes?

Protocol number: eb/lm/let390/ln950/20038

DATE:

Patient code:

Randomisation number:

TAXI : YES/NO

| | Date/time | Comments |
|------------------------|-----------|----------|
| Visit 1 (screening) | | |
| Visit 2 (week 0) | | |
| Visit 3 (week 2) | | |
| Visit 4 (week 4) | | |
| Visit 5 (week 8) | | |
| Visit 6 (6 months) | | |
| Visit 7 | | |

SCREENING VISIT (VISIT 1)

DATE:

MALE/FEMALE

Past Medical History:

Drug History:

ALLERGIES

Social History: Smoker/ Non-Smoker If yes amount and years:
Alcohol:

Family History:

EXAMINATION

Pulse rate:

BP:

Ankle Oedema:

JVP:

Height:

Weight:

BSA:

General remarks:

CVS:

RS:

CRITERIA:Diabetic ☐Possible LVH (ECG) ☐Office target BP <150/90 ☐**EXCLUSION CRITERIA:**Gout ☐Already on Allopurinol ☐Previous adverse reaction to Allopurinol ☐Poor kidney function (eGFR <60ml/min) ☐Conditions that exclude Magnetic Resonance Imaging (MRI) ☐Heart failure (LVEF <45%) ☐Cancer or other life threatening illness ☐Pregnancy or breast feeding (?pregnancy test) ☐Unable to provide consent ☐Atrial fibrillation (not definite exclusion criteria) ☐

INITIAL INVESTIGATIONS:

| | |
|---------------------------------|--------------------------|
| Consent | <input type="checkbox"/> |
| Letter to GP | <input type="checkbox"/> |
| File letter/consent in notes | <input type="checkbox"/> |
| Blood pressure | <input type="checkbox"/> |
| Information about study given | <input type="checkbox"/> |
| Echo report | <input type="checkbox"/> |
| Urea and electrolytes (U and E) | <input type="checkbox"/> |
| Liver function tests | <input type="checkbox"/> |
| Full blood count | <input type="checkbox"/> |
| HbA1c | <input type="checkbox"/> |

Date for next visit:

Any other comments:

ECHO

IVSd:

IVSs:

LVIDd:

IVIDs:

LVPWd:

LVPWs:

LV mass:

BSA:

MV:

AV:

TV:

PV:

General comments:

Signed:

Date:

VISIT 2 (week 0)**DATE:**

SCREENING BLOOD RESULTS CHECKED

☐

SYMPTOMS/ PROBLEMS SINCE LAST VISIT (inc. new drugs)

Uric acid

☐

Fasting glucose/insulin (HOMA index)

☐

HbA1c

☐

BNP

☐

PIIINP (collagen marker)

☐

Renin and aldosterone

☐

ECG

☐

Urine test

☐

Sphygmocor

☐

Flow mediated dilatation (FMD)

☐

Cardiac MRI organised

☐

24 hour blood pressure monitoring

☐**FIRST SET OF STUDY TABLETS** (Allopurinol 100mg/placebo)☐**Date for next visit:**

Any other comments:

Flow Mediated Dilatation

BP

GTN

Sphygmocor

PWA

PWV

Cardiac MRI

LVM

LVM Corrected

LA volumes

EF

ECG

Signed:

Date:

VISIT 3 (Week 2)**DATE:**

VISIT 2 BLOOD RESULTS CHECKED

☐

SYMPTOMS/ PROBLEMS SINCE LAST VISIT (inc. new drugs)

Urea and electrolytes (U and E), FBC, LFT

☐**SECOND SET OF STUDY TABLETS** (Allopurinol 300mg/placebo)☐**Date for next visit:**

Any other comments:

Signed:

Date:

VISIT 4 (Week 4)**DATE:**

VISIT 3 BLOOD RESULTS CHECKED

☐

SYMPTOMS/ PROBLEMS SINCE LAST VISIT (inc. new drugs)

U and E, LFT, FBC

☐

Urine test

☐**THIRD SET OF STUDY TABLETS (Allopurinol 600mg/placebo)**☐**Date for next visit:**

Any other comments:

Signed:

Date:

VISIT 5 (Week 8)**DATE:**

VISIT 4 BLOOD RESULTS CHECKED

☐

SYMPTOMS/ PROBLEMS SINCE LAST VISIT (inc. new drugs)

U and E, LFT, FBC

☐**Date for next visit:**

Any other comments:

Signed:

Date:

VISIT 6 (month 6)**DATE:**

VISIT 4 BLOOD RESULTS CHECKED

☐

SYMPTOMS/ PROBLEMS SINCE LAST VISIT (inc. new drugs)

U and E, LFT, FBC, uric acid

☐

Urine test

☐

Sphygmocor

☐

Flow mediated dilatation (FMD)

☐

Cardiac MRI booked for next visit

☐

BP:

Date for next visit:

Any other comments:

Flow Mediated Dilatation:

BP

GTN

Sphygmocor

PWA

PWV

Signed:

Date:

VISIT 7 (9 months) FINAL VISIT**DATE:**

VISIT 5 BLOOD RESULTS CHECKED

☐

SYMPTOMS/ PROBLEMS SINCE LAST VISIT (inc. new drugs)

Urea and electrolytes (U and E)

☐

Liver function tests

☐

Full blood count

☐

Uric acid

☐

Fasting glucose/insulin (HOMA index)

☐

HbA1c

☐

BNP

☐

PIIINP (collagen marker)

☐

Renin and aldosterone

☐

Urine test

☐

Sphygmocor

☐

Flow mediated dilatation (FMD)

☐

Cardiac MRI organised

☐

24 hour blood pressure monitoring

☐

BP:

STUDY TABLETS STOPPED☐

Any other comments:

Flow Mediated Dilatation

BP

GTN

Sphygmocor

PWA

PWV

Cardiac MRI

LVM

LVM Corrected

LA volumes

EF

Signed:

Date:

Blood results:

| | Week 0 | Week 2 | Week 4 | Week 8 | Month 6 | 1 year |
|----------------------------|--------|--------|--------|--------|---------|--------|
| Na | | | | | | |
| K | | | | | | |
| Urea | | | | | | |
| Creatinine | | | | | | |
| Hb | | | | | | |
| WCC | | | | | | |
| Plt | | | | | | |
| Bilirubin | | | | | | |
| AST | | | | | | |
| ALT | | | | | | |
| Alk Phos | | | | | | |
| Albumin | | | | | | |
| Uric acid | | | | | | |
| Fasting glucose/insulin | | | | | | |
| HbA1c | | | | | | |
| BNP | | | | | | |
| PIIINP | | | | | | |
| Renin | | | | | | |
| Aldosterone | | | | | | |
| Creatinine clearance | | | | | | |
| 24hour urine protein | | | | | | |
| Microalbuminuria | | | | | | |